Barnegat Bay—Year 2

Baseline Characterization of Phytoplankton and Harmful Algal Blooms

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Baseline Characterization of Phytoplankton and Harmful Algal Blooms in Barnegat Bay-Little Egg Harbor Estuary, New Jersey (Year Two)

FINAL REPORT

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EXECUTIVE SUMMARY

Barnegat Bay-Little Egg Harbor (BB-LEH) in New Jersey is very susceptible to human-induced eutrophication due to its shallow depth, relatively long flushing time and highly developed surrounding watershed. The Estuary has been classified as a highly eutrophic system (Nixon 1995, Bricker et al. 2007), experiencing episodic recurrences of brown tides and other microalgal blooms, loss of submerged aquatic vegetation, and decline of hard clam stock and harvest.

We carried out a two-year survey of the phytoplankton community in BB-LEH estuary in coordination with New Jersey Department of Environmental Protection (NJDEP)ôs Bureau of Marine Monitoring during 2011-2013. The study aimed to characterize species composition and spatial and temporal trends in the BB-LEH phytoplankton community, including bloom patterns, dominant species succession, and occurrence of Harmful Algal Bloom (HABs) species. Additional study goals were to compare year-to-year changes on phytoplankton community structure, and to understand the relationships between the changes of species composition and environmental variables. The Year-one report included the species composition of phytoplankton and its seasonal variations from 9 sites from August 2011 to September 2012 (Ren 2013).

This report presents the data on the phytoplankton community from October 2012 to August 2013, based on samples collected at monthly and biweekly intervals from 6 sites throughout BB-LEH. Analysis of phytoplankton included species identification and enumeration, and calculation of cell density and biovolume. In addition, the report includes the results from multivariate analyses on the temporal changes of phytoplankton between Year-one and Year-two, and the relationship between the phytoplankton changes and environmental conditions based on the Year-one and Year-two data.

The number of phytoplankton taxa recorded from the Year-two samples was similar to Year-one. Most common species belonged to five major groups: diatoms (Bacillariophyceae), dinoflatellates (Dinophyceae), cryptophytes (Cryptophyceae), chlorophytes (Chlorophyceae), and chrysophytes (Chrysophyceae). Diatoms made up approximately 50% of the total number of taxa, followed by dinoflagellates. There were differences detected between Year-one and Year-two in regards to species occurrence and dominance among seasons and sites, but species richness and diversity were comparable between the two years. Some species, such as the small and spiny diatom *Chaetoceros* cf. *tenuissimus,* which formed massive blooms in northern Barnegat Bay sites in spring 2012, were not observed at all in 2013. Diatoms were the major components dominating the phytoplankton biomass at all sites during winter-spring in 2012-2013. Picoplankton, pico-coccoids and co-occurring cyanobacteria were numerically dominant in summer at the sites north of Barnegat Bay inlet, coincident with Year-one. In southern Barnegat Bay and Little Egg Harbor, summer phytoplankton in Year-two was dominated by small centric diatoms and lightly silicified diatoms.

Less frequent occurrence and low abundance of harmful species were observed in comparison to Year-one. Major harmful species found in Year-two were *Heterocapsa rotundata* (=*Katodinium rotundatum*) in winter, and *Synechococcus* sp. in summer. For both species, the highest abundance was detected near the mouth of Toms River. *Prorocentrum minimum* was observed at high abundance (10^5~10^6 cells L^{-1}) in Northern Barnegat Bay in Year-one (winter-spring 2011/12), but was not encountered in Year-two. *Prorocentrum* species, including *P. minimum*, *P. triestinum* and *P. micans* were found in a few samples during the study period, but at lower cell density (10^4~10^5 cells L^{-1}) compared to Year-one. In addition, *Pseudo-nitzschia* species were detected in Little Egg Harbor in Year-one with occasional high abundance, but were not encountered in Year-two.

The inter-annual changes of phytoplankton species composition varied in different areas of BB-LEH, likely related to their specific hydrological conditions. In northern Barnegat Bay (BB01), where the water residence time is longest, the phytoplankton community was significantly influenced by Hurricane Sandy. Winter and spring phytoplankton assemblages after the Hurricane were significantly different compared to those from the previous year. The phytoplankton community at BB04 may have been more affected by Toms River with its freshwater discharge and nutrient loading. The summer and winter communities were similar between the two years. And spring and fall community changes were more dynamic, corresponding to temperature changes. In southern Barnegat Bay and Little Egg Harbor, as represented by BB09 and BB12, the phytoplankton community after the Hurricane was more or less distinct from that before the storm. Seasonally, the spring and fall changes were more dynamic compared to other seasons.

Multivariate analysis of all samples from Year-one and Year-two showed significant relationships between phytoplankton species composition and the environmental variables. In addition to salinity and temperature, several nutrient variables were significantly related to the change of phytoplankton community, including total nitrogen (TN), dissolved silica (DSi), total phosphorus (TP), TN:TP ratio, dissolved and total organic carbon (DOC and TOC), as well and dissolved oxygen (DO) and total suspended solids (TSS). High abundance of diatoms was negatively related to DSi in the water column in both years, indicating Si limitation in spring and summer. The dominance of pico-coccoids and cyanobacteria in summer was significantly related to high nutrients, particularly TN and dissolved organic matter, and low concentration of dissolved oxygen in the water column.

The results further confirmed that the change in species composition was sensitive to nutrient input in BB-LEH, and that the phytoplankton community is an important component of water quality monitoring. Our study provides valuable information for the development of indicator species. The two years of phytoplankton data, in combination with water quality data, provide a good starting point for the development of biotic indices for water quality assessment in BB-LEH. Furthermore, data from this study, which include species composition and abundance, biovolume, and carbon biomass, can be useful for related studies on understanding interactions between anthropogenic nutrient loadings, phytoplankton response and food web alteration in BB-LEH, and on modelling development for BB-LEH water quality management. The image and taxonomic documentation generated from this study provides valuable information on phytoplankton taxonomy and data comparison for future studies in BB-LEH and adjacent regions.

Uncertainties and Recommendations

The study showed significant year-to-year differences in phytoplankton assemblages and species succession at different sites in BB-LEH between 2011 and 2013. In addition to nutrient loading, precipitation/drought and hydrology are considered the important factors affecting the inter-annual changes in the phytoplankton community. The study showed that Hurricane Sandy had affected the phytoplankton composition in BB-LEH. How the resulting phytoplankton changes related to associated food web changes is beyond the current scope of work. Further detailed studies are recommended to link system alterations by extreme weather events with the changes of phytoplankton and other biological components, and the effects on system water quality for the long term. Analyses of indicator species are underway for the P-IBI development as part of the scope of work for Year-three. However, a phytoplankton index based on two years of data may inevitably exhibit uncertainty as the estuarine system was altered by the disturbance of the Hurricane. In addition, multivariate analysis including all-season data showed water temperature being a more important factor than most other variables. Future data analyses on data subset by seasons should be conducted to partial out temperature influences and focus more on nutrients and other water quality characteristics. Therefore, more investigations on seasonal variations in phytoplankton communities are recommended to better understand the development of the ecosystem after Hurricane Sandy, and to accumulate a longer term dataset for further qualitative and quantitative analysis of the associations among nutrient loadings, phytoplankton responses and other habitat characteristics, and indicator development.

Brown tide, the bloom of *Aureococcus anophagefferens*, has been a major concern in Barnegat Bay-Little Egg Harbor system. It has occurred episodically since the first confirmed detection in 1995. Like other algal blooms, its formation can be a result of many factors, including hydrological, meteorological, as well as chemical and biological conditions. Therefore, the blooms are usually patchy, and their breakdown can be rather rapid, which makes HAB monitoring a challege. In this study, we used the method of polyclonal antibody labeling and fluorescence microscopic observation and detected low density of *Aureococcus anophagefferens* in southern Barnegat Bay and Little Egg Harbor. An incidence of *Aureococcus anophagefferens* bloom, however, was detected near Sedge Island on June 19, 2013 (4.5×10^8 cells/L, Bricelj et al. unpublished data). In addition, several other HAB species were recorded in this study including *Prorocentrum minimum*, *Heterocapsa rotundata* (=*Katodinium rotundatum*), *Dinophysis acuminate*, *Pseudo-nitzschia* and *Chaetoceros* species which occurred at high cell density. Even though the detected species and their abundance varied year to year, the study showed their presence in BB-LEH, which is a primary factor indicating the potential for harmful blooms. Continuous monitoring and studies on these HAB species in BB-LEH, including their dominance and blooms and the triggering factors and mechanisms, are recommended.

INTRODUCTION

Background

Phytoplankton responds directly to changes in physical and chemical condition in aquatic systems. The direct effects of nutrient enrichment on phytoplankton in estuaries include excessive growth and biomass, species shifts and frequent noxious and harmful algal blooms (Glibert et al. 2005). These changes in phytoplankton components have significant effects on the organisms at higher trophic levels in the food web. Fish kills and/or reduction of some important fishery resources are often linked, directly or indirectly, to some specific algae, especially harmful algal blooms. The complex interactions between anthropogenic nutrient loadings, phytoplankton species composition, and higher trophic alteration are fundamental to understanding the ecological status of any estuarine and coastal systems.

The Barnegat Bay-Little Egg Harbor Estuary (BB-LEH) is a shallow, poorly flushed system bordered by a highly developed watershed. It is therefore very susceptible to nutrient enrichment. The Estuary has been classified as a highly eutrophic system as determined by application of the National Oceanic and Atmospheric Administration (NOAA) National Estuarine Eutrophication Assessment model (Bricker et al. 2007) and Nixonĝs Trophic Classification (Nixon 1995, Kennish et al. 2007). The ecological health of the estuary has deteriorated over the last few decades with episodic recurrences of brown tides and other microalgal blooms, loss of submerged aquatic vegetation, and decline of hard clam stock and harvest (Kennish et al. 2010a). A recent USGS report showed that total nitrogen (TN) and total phosphorus (TP) loading from surface runoff for the entire BB-LEH has been increasing, and ranged from 455,000 kg N (in 1995) to 857,000 kg N (in 2010), and from 17,000 kg P (1995) to 32,000 kg P (2010), respectively (Baker et al. 2014). On average, the northern segment accounted for more than half of the annual nutrient loads, 66% for TN and 63% for TP, due to the highly developed watershed. Understanding the relationships between the ongoing nutrient loading (including the forms, concentration and ratios of nutrients) and the changes of phytoplankton community is essential for water quality assessment and management in the BB-LEH Estuary.

We investigated the phytoplankton community in BB-LEH from August 2011 to September 2012 within the scope of work in the Year-one project. Species composition and cell density, seasonal changes of dominant/abundant species, and species succession from nine sites throughout the BB-LEH region were studied and recorded in detail. Several major HAB species and their occurrences and abundance were documented, including some potentially harmful species detected in the north at high abundance. They had not been recorded before this study (Ren 2013). Previous studies as well as monitoring data showed that year-to-year change of phytoplankton community and bloom development varied greatly in BB-LEH, especially concerning harmful algal blooms and brown tide occurrences (Schuster 1999, 2004, Pecchioli et al. 2006). In order to better understand annual and inter-annual changes of phytoplankton in BB-LEH, continuous investigation on the phytoplankton species composition and abundance was carried out at six sites from October 2012 to August 2013, using a monthly-biweekly sampling strategy.

Objectives of Study

The Year-two study aims to 1) continue to characterize phytoplankton species composition and its spatial and temporal trends in the BB-LEH (2012-2013); 2) document bloom patterns and dominant species successions over time (2012-2013; 3) compare year-to-year changes on phytoplankton community structure (2011-2013); and 4) understand the correlations between the changes of species composition and environmental variables (over the two-year study period. In addition, same as in Year-one, we calculated biovolume biomass based on species abundance and biovolume measurements. We further calculated carbon biomass based on biovolume measurements. The overall objective of the study is to provide baseline information on the phytoplankton community in BB-LEH to assist the assessment of current water quality and the development of management tools.

FIELD AND LABORATORY METHODS

Sampling

Cluster analysis based on Year-one phytoplankton community data showed that the 8 sites in BB-LEH can be classified mainly into three groups (Fig. 1). Among all the sites, BB01, BB02, BB05a and BB07a in northern Barnegat Bay can be grouped together, and B09, BB12 and BB14 can be grouped together. BB04a, near the mouth of Toms River, is in general different than the other sites. BB12 and BB14 in Little Egg Harbor were clustered together closely. Accordingly, in the Year-two investigation, six sites, BB01, BB04a, BB07a, BB09, BB10 and BB12, were selected for continuous phytoplankton community analysis. The selected sites span a range of salinity and nutrient regimes along BB-LEH. The locations of the sites are shown in Table 1 and Fig. 1. In particular, BB01 is located at the northernmost end of Barnegat Bay, just south of the Mantoloking Bridge and a USGS monitoring site (USGS01408168). BB04a is located near the mouth of Toms River. BB07a and BB09 are located north and south of Barnegat Bay Inlet. BB10 is located near the adjunction of Barnegat Bay and Little Egg Harbor. And BB12 is located within Little Egg Harbor. All six sites were coordinated with the existing sites of the NJDEP water quality monitoring in BB-LEH (Barnegat Bay LMP QAPP 2013). In year-two, phytoplankton samples were collected at the above-mentioned sites from October 2012 to August 2013. In total, 79 samples were collected from these 6 sites and analyzed quantitatively for phytoplankton species composition.

Phytoplankton sample collections were synchronized with NJDEP water quality grab samplings, and were done via the courtesy of NJDEP Bureau of Marine Water Monitoring. Surface water (<0.5 m) was collected monthly from October 2012 through March 2013, and biweekly from April through August 2013. The samples were preserved with glutaraldehyde to a final concentration of about 0.5%-1% (v/v). Samples were kept dark and cool (~ 4^{o}C) during transportation and prior to analysis. For each sample, three different processes were performed, 1) about 150-250 ml of sample water was dispensed for size-fractionated filtration and whole-community microscopic analysis; 2) one-liter of sample water was settled for further processing for diatom analysis when necessary; 3) about 200-500 ml of water, depending on the biomass, was settled to concentrate to about 20 ml for qualitative and light microscopic observation, if necessary, and for archive purposes. The remnants from 1) were also kept for archive.

Phytoplankton Whole-Community Counts

Phytoplankton samples were size-fractionated by filtering through 0.2 µm, 3 µm and 8 µm pore-size filters. The latter two fractions were stained with 0.03% proflavine hemisulfate. The 0.2 to 3 µm fraction was counted immediately after filtration. The >8 µm fraction was frozen and counted later. Algal identification and enumeration, including soft-algae and diatoms, were done under an epifluorescence microscope (Leica DM L) with blue and green excitation lights and transmitted light. For 0.2 and 3 μm pore-size filters, observations were done under ×1000 magnification. For each filter, at least 5 random fields were counted or until at least 100 cells were counted. If the filter was very sparse, then 50 random fields were counted before stopping. For 8 μm pore-size filters, each filter was observed under three magnifications: First, under ×1000 magnification for phytoplankton <20 μm with the same counting strategy in terms of finishing point; second, under ×400 magnification for larger (>20 μm) phytoplankton with a maximum of 25 random fields when it was sparse; Third, under ×100 magnification to catch some large organisms, which might not have been able to be counted under higher magnifications due to either their large size or sparse density. The method allowed us to be able to examine small size phytoplankton (< 20 μm) under higher magnification (×1000) compared to other methods, e.g. using Palmer-Maloney and/or Sedgewick-Rafter counting cells. The blue and green excitation helps us to differentiate groups of algae when stained with dyes (Dortch et al. 1997, Ren et al. 2009). For samples with high abundance and diversity of diatoms, diatom slides were made separately. Diatoms were analyzed to get the percentage of dominant diatoms, especially the small centric diatoms. Phytoplankton species were identified to the lowest taxonomic level possible. In addition, each common taxon (5% of total cell counts) was documented with images. Biovolumes of common taxa were calculated based on microscope measurements of dimensions and geometric models of phytoplankton (Hillebrand et al. 1999, Olenina et al. 2006). Carbon biomass was calculated based on the biovolume measurements and the cell carbon content for diatoms and non-diatoms from literature (Eppley et al. 1970).

Multivariate Analyses

Cluster analysis

Cluster analysis, using Wardǿs linkage method and Euclidean distance measure, was performed to find groups for classification of sites and seasons with respect to phytoplankton community changes. For Year-one analysis, since three sites (BB04, BB05 and BB07) were shifted after 05/23/2012 (see more information in Ren 2013), in order to exclude the effect of location shifting, only samples collected after the 05/23/2012 were used in the cluster analysis for site classification. Cluster analysis for season classification was conducted on those sites with continuous sample collection from the beginning. Outlier analysis was performed prior to cluster analysis for each dataset.

Nonmetric Multidimensional Scaling

Nonmetric Multidimensional Scaling (NMDS, or NMS) was conducted in the study to establish seasonal and year-to-year changes in phytoplankton community using two years of data from August 2011 to August 2013. The method is an ordination technique, calculating the similarity (dissimilarity) in species composition among each pair of samples. Different from the other classical scaling (e.g. Principal coordinates analysis), NMDS considers only the rank order of the dissimilarity (similarity) instead of their quantitative values. In addition, instead of performing eigenvalue calculations, NMDS uses iterative least-squares stress fitting to obtain the final case configuration from an initial one. Therefore, the number of axes (dimensions) must be specified in advance to final NMDS. Two steps were thus taken for each NMDS analysis, the preliminary runs, and a final run. Preliminary runs were done on each dataset, as the first step, to select the number of dimensions and final stress and to check the final stability (PC-ORD 4.5, McCune and Grace 2002). The information was then used in the second step, the final run of NMDS which was done in Canoco 5. Sample distances were calculated using Bray-Curtis distance measure. One characteristic of NMDS is that it has no precise analytical solution. In Canoco 5, the case configuration was gradually optimized and obtained using an iterative procedure. For this study, the solution of principal coordinates analysis (PCO, also known as metric multidimensional scaling) was used as the starting configuration with no random distortions (perturbations). The variation of case scores along NMDS axes was then maximized by applying a principal component rotation to the scores resulting from the NMDS optimization process (ter Braak and Ġmilauer 2012). A map of the samples was constructed in two or more dimensions, in which relative distance apart of the samples reflects relative similarity in species composition. There has been increasing use of NMDS in recent studies related to biological community changes in aquatic ecosystems (Clarke 1993, Rothenberger et al. 2009, Reynolds et al. 2014).

Principal Components Analysis

Principal Components Analysis (PCA) was performed to seek correlations among environmental variables. This step was used to reduce the number of variables in the water quality dataset but without losing much of the information that was in the original dataset. Solutions based on proportionately more variables will be less stable and the resulting eigenvector coefficients will be less reliable (King and Jackson 1999). More than 20 physical and chemical variables were measured and analyzed in BB-LEH from the NJDEP Water Quality Monitoring Project since summer 2011. More information can be found at http://www.state.nj.us/dep/barnegatbay/bbmapviewer.htm.

Water quality data from the same dates as phytoplankton collections were extracted for this study. Several variables were excluded prior to the data analyses because more than 3/4 (arbitrarily set) of the data values were missing or below limit of detection (LOD). For this reason, variables such as nitrate+nitrite (NO3+NO2), NH3, and ortho-P were excluded from the environmental matrix. For the variables with only few missing data, the following strategies were used to fill in the missing or below LOD data, so that information could be kept: 1) the values below LOD were arbitrarily set to half the detection limit as recommended in several references (Hornung and Reed 1990, Lambert et al. 1991). For missing data, values were artificially set either with the mean values from nearby sites or those from the same month. In addition, TN:TP ratios (in moles) were calculated from TN and TP and included in the environmental matrix. In the end, there were 17 environmental variables for Year-one (Table 2), and 21 for Year-two PCA analyses (Table 3).

Canonical Correspondence Analysis

Canonical Correspondence Analysis (CCA) was conducted to explore the relationship between the phytoplankton species community and environmental factors. For Year-one, phytoplankton community data included samples collected from September 2011 to September 2012. Rare taxa (<5% of total abundance) were excluded in the species matrix prior to the analysis. For Year-two, phytoplankton community data included the samples collected from October 2012 to June 2013. Although phytoplankton species data in Year-two were analyzed through August 2013, water quality data were available through June 2013. All taxa were included in the species matrix, but rare taxa were down weighted during analysis. For both Year-one and Year-two analyses, the species abundance was log transformed prior to the analyses. A few variables were excluded in environmental data matrix because of their strong correlations to other variables, in particular, dissolved oxygen (DO) saturation to DO concentration, alkalinity and conductivity to salinity. In the end, 13 environmental variables were included in Year-one CCA analysis and 11 variables in the Year-two CCA analysis. Datasets for CCA analyses are summarized in Table 4.

Cluster analysis, PCA and first run of NMDS were carried out using PC-ORD 4.5 (McCune and Grace 2002). Final run of NMDS and CCA analyses were performed in Canoco 5.0 (ter Braak and Ġmilauer 2012).

RESULTS AND DISCUSSION

Physical and Chemical Conditions

Water quality monitoring data from October 2012 to June 2013 were downloaded from a NJDEP website: http://www.nj.gov/dep/barnegatbay/bbmapviewer.htm. The variation of water temperature, salinity (ppt), Secchi depth (ft), total nitrogen (TN), total phosphorus (TP) and dissolved silica on the dates of phytoplankton collections from the six sites are illustrated in Fig. 2 and Fig. 3.

There was little difference in water temperature among the six sites (Fig. 2). The lowest temperature was detected in January- February and the highest in late June (August data were not shown). Salinity did not show much seasonal variation and fluctuated from 22 to 30 ppt for most of the sites. Salinity at BB01 and 04a was relatively low and exhibited larger fluctuations compared to the other four sites. The highest salinity at BB01, close to 30 ppt, was detected in November 2012, shortly after the landfall of Hurricane Sandy (Oct. 29, 2012). Salinity at BB04a was the lowest among all sites with the lowest value detected in December 2013, indicating freshwater input from Toms River. Secchi depth, a measure of water clarity, fluctuated between 2 and 6 ft at all sites, not showing much seasonal variation and gradient among different sites.

Recent study showed a strong gradient in watershed nutrient loading, from northern to southern segments (Baker et al. 2014). The northern segment, on average, accounted for over 60% of total annual TN and TP loading, while central and south segments accounted for <20%. However, the changes of TN and TP in BB-LEH did not show much spatial gradient from north to south during the study period. Seasonally, slightly lower concentrations of TN and TP were detected from December 2012 to May- June 2013 compared to other months. Dissolved silica (DSi) exhibited similar seasonal trends to TN and TP. For most sites, the concentration of DSi was low from December 2012 to June 2013, especially in March-April when DSi values were often below the limit of detection (0.01 mg/L). The concentration of DSi increased gradually in summer at all sites. At BB04a, the concentration of DSi showed a spike in December 2012, and because of the replenishment, the concentration of DSi at BB04a was generally higher most of time in comparison to other sites until March-May 2013 when it reached the lowest.

Correlation coefficients among major environmental variables were shown in Table 3. Salinity was highly correlated with conductivity and alkalinity. Turbidity (in TNU) was significantly correlated with total suspended solids (TSS), and Secchi depth measurements. The concentrations of TN, dissolved nitrogen and nitrate+nitrite (NO3+NO2), as well as TOC and DOC were negatively correlated with salinity, indicating freshwater loading, mostly from surface water runoff and groundwater discharge, which was a significant source for nitrogen and organic carbon in the BB-LEH (Wienben and Baker 2009). TP and dissolved P, despite its source from freshwater loading (Baker et al. 2014), had less correlation with salinity, but were more significantly related to temperature and Secchi depth and TSS, possibly suggesting the dominant control of TP and dissolved P concentration in BB-LEH may be biological processes.

Species Composition and Seasonal Changes

A total of 136 taxa were recorded from the Year-two study, and most of the common ones had been recorded from Year-one (Ren 2013). Same as in Year-one, most common species belong to the following five major groups: diatoms (Bacillariophyceae), dinoflatellates (Dinophyceae), cryptophytes (Cryptophyceae), chlorophytes (Chlorophyceae), and chrysophytes (Chrysophyceae). Diatoms comprised the largest number of species, 66, or 50% of the total taxa, followed by dinoflagellates with 24, or 18% of the total. The following diatoms were common at most sites: *Cyclotella* species including *Cyclotella choctawhatcheeana* and *C. atomus*, *Skeletonema 'costatum'*, *Dactyliosolen fragilissimus* and several *Chaetoceros species*. Diatoms, *Asterionellopsis glacialis*, *Leptocylindrus minimus*, *L. danicus*, *Thalassiosira* spp., and *Cylindrotheca closterium* were more often found in middle and southern sites in BB-LEH (BB07, BB09, BB10 and BB12). In addition, some taxa such as *Minutocellus scriptus* (in Year-one, *Minutocellus* sp. 1), *Minidiscus* and *Skeletonema menzelii* emerged in summer at southern sites (BB10, BB12). Main dinoflagellates observed from Year-two included *Gyrodinium flagellare*, *G. estuariale, Heterocapsa triquetra* and *Heterocapsa rotundata* (=*Katodinium rotundatum* in Year-one). They were more often detected in winter-spring and summer at relative low abundance (10^4~$10^5 L^{-1}$) compared to Year-one, except for *H. rotundata*. The highest abundance of *H. rotundata* was found in November at BB04a, > 1.0 x10^6 L^{-1}. High abundance of

Prorocentrum minimum observed from Northern Barnegat Bay in winter-spring 2011/12, did not occur in winter-spring 2012/13. *Prorocentrum,* including *P. minimum*, *P. triestinum* and *P. micans* were found occasionally during the study period, but at very low cell density (10^3 L^{-1}). Cryptophytes have been a consistent component of phytoplankton community during most of the year in BB-LEH. The common taxa included *Hemiselmis* spp., *Plagioselmis* sp., *Teleaulax acuta* and *Leucocryptos marina*. The cell density of major cryptophytes varied with season, and were abundant in spring and summer as observed from BB01, BB09 and BB10 (Fig. 4, Fig. 7 and Fig. 8). Crysophytes were another consistent part of phytoplankton in BB-LEH, mainly comprised of *Calycomonas ovalis*, *C. gracilis*, and *Pseduopedinella pyriformis*. Cyanobacteria became dominant, numerically, in summer (June to August) and in the northern region. Except for the coccoidal cyanobacterium *Aphanocapsa* sp., which had been recorded from Year-one, a skinny oscillatorial *Planktolyngbya* species was observed from most of the sites in April/May. In addition, it is worth mentioning, because of its potential harm, *Synechococcus* sp. was detected at BB04a in May. The typical picoplankton, pico-coccoids being consistent with Year-one, was again detected in summer (June and August) and early fall (October) from sites BB01, 04a, 07a and 09, while at southern sites (BB10 and 12) it was detected in May, but at relatively low density (10^7 L^{-1}).

Cluster analysis with preliminary trial based on all samples from all sites did not show good separation in terms of sites (not shown), possibly because the community dataset contained those from a few months after the Hurricane Sandy when the water system was mixed by the storm. Cluster analysis based on the samples from each site was performed, and showed approximate seasonal separation for most of the sites. Overall, three season groups may be indicated for BB01, BB04a and BB07a: November-December-January/February (winter), March-April-May (spring), and May-June-August (summer). But for southern sites (BB09, BB10 and BB12), the separation could be from November-April, and from April-August. At BB01, 04a and BB07a, the diatoms *Cyclotella choctawhatcheeana, S. costatum* and *Chaetoceros* dominated winter and early spring assemblages. In spring, diatom *D. fragilissimus* started to grow and became dominant in late spring and early summer, while cryptophytes were also abundant. From June to August, the dominant species succession was small size of *Cyclotella atomus*, Pico-coccoids and then cyanobacteria in August (Fig. 4-6). At sites BB09, BB10 and BB12, phytoplankton assemblages were dominated by diatoms during the most of the study period. The succession of the abundant species was as follows: *S. costatum*, *Chaetoceros* spp. *A. glacialis* and *Leptocylindrus* spp in winter-spring →*Thalassiosira* spp., Cryptophytes and Chrysophytes in summer→ small diatoms *Th. Proschkinae*, *Skeletonema menzelli* and *Minutocellus scriptus* in August (Fig. 7-9).

Calculated biovolumes of major phytoplankton taxonomic groups showed that diatoms dominated the phytoplankton biomass from November to May and pico-coccoids dominated the other months at BB01, 04a and 07a (Fig. 10-12). Cyanobacteria, co-occurring with pico-coccoids, although numerically abundant, did not contribute much because of small cell sizes. Diatoms accounted for the majority of phytoplankton biomass at the sites BB09, 10 and 12 during the study period (Fig. 13-15).

Chlorophyll *a* is a routine parameter in water quality monitoring for estimating overall algal biomass. However, knowing the variation and distribution of phytoplankton carbon is important for better understanding carbon dynamics in coastal ecosystems, and for modeling development because carbon is one general currency in biological models (Glibert et al. 2010). Unfortunately, unlike chlorophyll *a*, there is no direct in-situ measurement for phytoplankton carbon biomass, and it is usually estimated from cell biovolume through the microscopic measurements (Mullin et al. 1966, Eppley 1970). The relationship between chlorophyll *a* and carbon biomass varies among different species, sometimes even the same species, but under different physiological conditions due to temperature, light and nutrient stress. It also varies with season in the same region because of different species compositions (Verity et al. 1992). From this study, a significant linear relationship between chlorophyll *a* measurement and calculated carbon biomass was obtained based on Year-two data (Fig. 16).

Year-to-Year Variations in Species Composition

Results from NMDS analysis considering phytoplankton species composition and cell abundance of both Year-one and Year-two samples from four sites were shown in two types of plots for each site of BB01, BB04 (04a), BB09 and BB12: sample scatter plot and sample-species biplot (Fig. 17-20). In both plots, the relative distance between the sample symbols reflects the similarity (points close together) or dissimilarity (points apart) in phytoplankton assemblages (their score values). Arrows between sample dots in sample scatter plots indicate the time series of sample collections. The red arrows mark (approximately) the landfall of Hurricane Sandy

(Oct. 29, 2012). In sample-species biplots, species which contribute most in determining the dissimilarity between groups were superimposed on the ordination diagrams. Each species arrow points in the direction of the steepest increase of species values. The angle between arrows indicates the correlation between individual species.

At BB01, the distance between October and November 2012 indicated large dissimilarity in phytoplankton community before and after the Hurricane at BB01 (Fig. 17a). Phytoplankton assemblages in winter and spring (November to May) following the landfall was significantly distinct from those at the same time in Year-one. The species succession in Year-one proceeded, from winter to spring, from dinoflagellate *Prorocentrum minimum* with diatom *S. costatum* → small Chlorophycean flagellate (*Chlamydomonas* sp. C)→Spine-forming *Chaetoceros*, whereas in Year-two Phytoplankton community was dominated successively by *S. costatum*, Chaetoceros and C. *choctawhatcheeana* from winter to spring. But *A. glacialis*, *D. fragilissimus* and *Leptocylindrus* spp. emerged as influential taxa accounting for the dissimilarity (Fig. 17b). Most of those diatoms, except for *C. choctawhatcheeana* had been detected in Year-one from southern sites with higher salinity. It is evident that the phytoplankton community at BB01 was affected by Hurricane Sandy which pushed more salt water to that part of Barnegat Bay, as shown by salinity in the November-December of 2013 (Fig. 2). Salinity dropped back to pre-hurricane level (~20 ppt) in January. However, most diatoms remained abundant through March-April, particularly *D. fragilissimus,* which became dominant but looked pale and stressed in April. Phytoplankton community in summer (June to August 2013) aggregated more into summer 2012, showing more similarity in phytoplankton between these two years, comprised of pico-coccoids, small *Cyclotella* species and cyanobacteria (Fig. 17a).

At BB04, summer and winter communities were similar between these two years, as shown by congregated samples in Fig. 18. But the changes were more dynamic during spring (such as, from March to June in 2013 as indicated by 1303 to 1306 in Fig. 18) and fall (October-November-December in 2012 as indicated by 1210-1212 in Fig. 18), corresponding to the change of temperature. High dissimilarity in phytoplankton between the month before and after the Hurricane was indicated, but it was not exceptionally high compared to the changes among other months (e.g. November to December, or March to April). The massive blooms of the small green alga *Chlamydomons* sp. *C* and the small diatom *Chaetoceros*, *Ch*. cf. *tenuissimus* recorded in spring and summer of Year-one, were not observed in Year-two, which largely contributed to the dissimilarity, especially in June for *Ch*. cf. *tenuissimus* (Fig. 18, lower panel). Different to BB01, phytoplankton community changes at BB04 were more likely affected by freshwater discharge, as well as nutrient loading, from Toms River. The Hurricane must have pushed the high salinity water to the area as it did to BB01. However, when the collection took place three weeks later, salinity fell back to the same range as before the storm. Variations in freshwater discharge as well as the concentration, forms and ratios of nutrients and organic matter in discharge will surely affect seasonal and inter-annual changes of phytoplankton growth and species composition at this site.

At BB09 the phytoplankton community after the Hurricane was distinct from that before the Hurricane (Fig. 19, Fig. 20). The site is located right below the Barnegat Bay Inlet. The change of phytoplankton was more dynamic, likely resulting from fast water exchanges with coastal water via Barnegat Bay Inlet (Defne and Ganju 2014). BB12 is located in Little Egg Harbor. The difference in the phytoplankton community between the two years was not as large as in BB09, but still evident (Fig. 20). Within each year, the change of the community was larger during spring and fall in comparison to winter and summer, correlating with temperature.

Variations in Species Composition in Relation to Environmental Variables

Sample-environmental variable biplots and species-environmental variable biplots (both axes 1 and 2), with score scaling focused on species scores, were generated from CCA, considering all data from Year-one and Year-two (Fig. 21 and Fig. 22). In the sample-environmental variable biplot, the distance between the sample symbols indicates the dissimilarity (similarity) of their species composition as measured by their chi-square distance. Environmental variables are represented by arrows in the plots. Each arrow points in the direction of the steepest increase of that environmental variable value (ter Braak and Smilauler 2012).

Significant relationships between phytoplankton species and the environmental variables were obtained in Year-one and Year-two, as indicated by Permutation tests with the results on all axes of P=0.002. The CCA explained a cumulative 79% and 68%, respectively, of the variation in the species-environmental relationships in Year-one and Year-two as shown by explained fitted variations in Table 5. The variables explaining most variation in phytoplankton community changes in Year-one were salinity, TN, water temperature, dissolved silica, DOC, DO, and TP (Table 5). In Year-two, the most explanatory variables included water temperature, DO, dissolved Si, TOC, TN and TP (Table 6). Salinity was the strongest variable in Year-one correlated with the change in species composition; however, it became a much less controlling factor in Year-two. Samples in Year-two were more congregated compared to Year-one, suggesting overall less temporal and spatial variation in species composition and, probably indicating the system was mixed due to Hurricane Sandy.

The species ï environmental variables diagrams summarize the variation of species composition in relation to the environmental variables. The 30 species with highest weight were shown. Salinity was the factor explaining most of the variation in phytoplankton abundance and species composition in Year-one. Diatom species showed strong correlation with the salinity gradient, with C. *choctawhatcheeana* and small *Chaetoceros cf. tenuissimus* in the spring in the northern area, as indicated by intermediate temperature and low salinity in the diagram. As salinity increased (toward southern sites), diatom assemblages were abundant with *Cerataulina pelagica*, *A. glacialis*, *Thalassionema*, *Leptocylindurs minimus*, *Skeletonema costatum* and small *Cyclotella stomus*. In summer and early fall, phytoplankton was dominated by pico-coccoids and cyanobacteria in northern part with relatively low salinity, while the diatoms *Thalassiosira proschkinae* and *Cylindrotheca closterium* were abundant in southern part of Barnegat Bay and Little Egg Harbor.

In Year-two, diatom distributions along the salinity gradient were still exhibited but relationships were not as strong as in Year-one. Due to the water mixing caused by Hurricane Sandy, the distribution of phytoplankton species did not show as much gradient from north to south in winter and spring in Year two. Species like *D. fragilissimus*, *A. glacialis*, *Thalassiosira* spp. had been detected abundant in northern area. Temperature emerged as the factor most closely associated with community changes, which was expected as the dataset included seasonal changes.

In both years, high abundance of diatoms was associated with lower dissolved Si (DSi) in the water column in winter and spring. This is not uncommon in estuarine ecosystems as shown by other studies (Conley and Malone 1992, Rothenberger et al. 2009). Dissolution of biogenic silica, mainly from diatom frustules buried in the sediment, can be a significant source for silicon restoration in the water column in shallow estuarine systems like Barnegat Bay (Loucaides et al. 2008). During the winter-spring period, regenerated Si was rapidly taken up by diatom populations; therefore DSi remained low. When temperature increased from spring to summer, the regenerated Si may not have been sufficient to match the increasing demand for Si from growing diatom populations. As a result, diatom growth was suppressed due to Si limitation. In the meantime, pico-coccoids and small coccoidal cyanobacteria, with no need for Si, grew rapidly. In addition, they could absorb and take up nutrients more efficiently under low N and P concentrations because of their size and shape (Gobler et al. 2011), which enables them to outcompete large-cell or more heavily silicified diatoms and became dominant in summer and early fall. Diatoms associated with summer picoplankton were found mostly to be small centric diatoms such as *Cyclotella atomus*, *Th. proschkinae,* and very lightly silicified ones, such as *Minutocellus scriptus* and *Phaeodactylum ? triconutum*. Dissolved Si in the water column was gradually accumulated in summer while diatom uptake decreased, as indicated by the positive relationship between dissolved Si and pico-coccoids/cyanobacteria abundance. One major consequence of excessive nutrient input, mainly N and P, in estuarine and coastal areas is the increase of Si limitation (Turner et al. 1998, 2003). It is one of the driving factors to promote a shift in dominance from diatoms to flagellates and cyanobacteria, as well as other non-diatom algae, some of which can be harmful to other organisms and water quality.

The phytoplankton community was significantly related to concentrations of TN and TP (Fig. 21, 22). The dominance of pico-coccoids and cyanobacteria in summer and its correlation with high TN and dissolved organic matter and low dissolved oxygen is coincident with other studies (Rothenberger et al. 2009, Gobler et al 2011). These studies showed that brown tide alga, *Aureococccus anophagefferens*, with similar size and shape as pico-coccoids in this study, outcompete co-existing phytoplankton at elevated levels of dissolved organic matter and turbidity and low dissolved inorganic nitrogen (Gobler et al. 2011). During the Year-one study, we detected low density (10^5~10^6 cells L^{-1}) of *A. anophagefferens* from sites 9 and 12. However, it did not shown up in the counts due to its low abundance. The dominance of dinoflagellates in winter-early spring, such as *P. minimum* in year-one, and *H. rotundata,* was coincident with some previous studies of Barnegat Bay and other mesohaline regions (Springer et al. 2005, Mountford 2013). The occurrence of *P. minimum* in Year-one was positively related to TN:TP ratios.

SUMMARY AND CONCLUSIONS

We investigated the temporal and spatial distribution of the phytoplankton community in BB-LEH from October 2012 to August 2013. We compared changes in phytoplankton assemblages between year-one (August 2011-September 2012) and year-two (October 2012-August 2013), and explored the effects of a variety of environmental variables on species composition. Significant relationships between phytoplankton community and environmental variables were indicated from both year-one and year-two analyses. Water quality parameters including TN, TP, dissolved Si, TN:TP, TOC and DOC, TSS and DO are strongly associated with the variation of phytoplankton species composition. The results further confirmed that the change in species composition was sensitive to nutrient input in BB-LEH, and that phytoplankton community is an important component of water quality monitoring. Our study provides valuable information for the development of indicator species. Furthermore, the two years of data, in combination of water quality data, provide a good starting point for the development of biotic indices for water quality assessment in BB-LEH. The inter-annual changes of phytoplankton species composition was significantly influenced by Hurricane Sandy. Our study provided baseline information on phytoplankton composition in the post-hurricane BB-LEH ecosystem. In addition, data from this study, which include species composition and abundance, biovolume and carbon biomass can be useful for other related studies, especially for the development of BB-LEH ecosystem models.

Major Findings of This Study

Diatoms became the major components of the phytoplankton community and biomass, and were numerically abundant all sites during most of the study period. Most diatom species recorded from year-two had been found in year-one samples. There were detected differences between Year-one and Year-two in regards to the species occurrence and dominance among seasons and sites, but the species richness and diversity were comparable between these two yeas. Some species, such as small and spiny diatom *Chaetoceros* cf. *tenuissimus* forming massive blooms in northern Barnegat Bay sites in spring 2012, were not observed at all in 2013. Despite the similar number of dinoflagellate taxa we recorded, their frequency and abundance were relative low compared to year-one. High abundance of *Prorocentrum minimum* observed from Northern

Barnegat Bay in previous winter did not occur in Year-two. But *H. rotundata* found in November at BB04a reached the cell density of > 1.0 x10^6 L^{-1}.

Cluster analysis of the samples from each site showed seasonal changes in species composition for most of the sites. For BB01, BB04a and BB07a, the development of phytoplankton community could be separated into winter (November-December-January/ February), spring (March-April-May), and summer (May-June-August). The dominant species proceeded from a mixture of *C. choctawhatcheeana, S. costatum* and *Chaetoceros* → *D. fragilissimus* and cryptophytes→ Pico-coccoids, Cyanobacteria and small *Cyclotella* species. For southern sites (BB09, BB10 and BB12), the seasonal separation was shown between November-early April and late April-August. The abundant species in the first half of the study period were a mixture of several diatoms, including *S. costatum*, *Chaetoceros* spp. *A. glacialis*, *Leptocylindrus* spp and *Thalassiosira*. In the second half of the period, phytoplankton in abundance was the small and lightly silicified diatoms *Th. proschkinae*, *Skeletonema menzelli* and *Minutocellus scriptus*.

Ordination analysis showed that phytoplankton community composition was significantly influenced by Hurricane Sandy. The largest change in the phytoplankton community was found at BB01 where the water residence time is the longest. Consequently, the 2013 winter and spring phytoplankton assemblages after the Hurricane were significantly different than those from the previous year. The phytoplankton community at BB04 may have been more affected by Toms River with its freshwater discharge and nutrient loading. In southern Barnegat Bay and Little Egg Harbor, as represented by BB09 and BB12, the change of the phytoplankton community was more dynamic, influenced by water exchange via Barnegat Bay inlet.

Multivariate analysis of all samples from Year-one and Year-two showed significant relationships between phytoplankton species composition and the environmental variables. Salinity appeared to be the most important variable in Year-one controlling the distribution of the phytoplankton composition; however, it became a much less important factor in Year-two. This difference may have been influenced by Hurricane Sandy, when more salt water was pushed to the north of BB, and retained for a considerably long time due to low turnover rate in the area. In addition, temperature was one of the strongest variables, which was expected in the all-season dataset. Several nutrient variables were significantly related to the change of phytoplankton community, including TN, dissolved silica, TN:TP, DOC, DO, and TP in year-one, and DO, dissolved Si, TOC, TN and TP in year-two. The distribution of diatoms showed a strong relationship with the salinity gradient in year-one. High abundance of diatoms was associated with lower dissolved Si in the water column in both years, indicating Si limitation in spring and summer in the system. The dominance of pico-coccoids and cyanobacteria in summer was significantly related to high nutrients, particularly TN and dissolved organic matter, and low concentration of dissolved oxygen in the water column.

Recommendations for Future Studies

The algal dataset, together with water quality monitoring data, provides us a good and ideal starting point to identify indicator species and develop a phytoplankton index of biotic integrity (P-IBI) for BB-LEH. Analyses of indicator species are underway for the P-IBI development, as part of the scope of work for Year-three of the NJDEP sponsored Barnegat Bay Research Program. Meanwhile, data analyses showed significant year-to-year differences in phytoplankton assemblages and species succession, likely due to effects of Hurricane Sandy. As a result, a phytoplankton index based on two years of data alone may inevitably exhibit uncertainty because the estuarine system was altered by the disturbance of the Hurricane. In addition, future data analyses on data subset by seasons should be conducted to partial out temperature influences and focus more on nutrients and other water quality characteristics. Further investigation and monitoring of phytoplankton and harmful algal blooms are therefore recommended to better understand and quantify the relationships between phytoplankton community change and the environmental factors, especially nutrients, in the post-Sandy BB-LEH system. For future analysis, it is necessary to include other factors in the analysis, such as watershed development, land use, freshwater discharge, precipitation, and turnover rate, in order to better understand temporal, spatial and inter-annual changes of the phytoplankton community in BB-LEH.

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LIST OF TABLES

Tables

Table 1. Sites of phytoplankton sample collection in Barnegat Bay-Little Egg Harbor from October 2012 to August 2013 (highlighted).

Site ID	Longitude	Latitude	Site description
BB01	-74.052222	40.04	Barnegat Bay at Mantoloking
BB02	-74.09847	39.97762	Barnegat Bay between Silver Bay and Goose Creek
BB04a	-74.14069	39.93289	Barnegat Bay near the Mouth of Toms River
BB05a	-74.1094237	39.9157764	Barnegat Bay above Cedar Creek
BB07a	-74.1571172	39.8012861	Barnegat Bay below Oyster Creek and above Barnegat Inlet
BB09	-74.14792	39.74262	Barnegat Bay below Barnegat Inlet and close to Long Beach
BB10	-74.20653	39.66095	Barnegat Bay by Route 72 Bridge
BB12	-74.26875	39.58151	Barnegat Bay in Little Egg Harbor
BB14	-74.29737	39.51123	Little Egg Harbor Inlet near Beach Haven Heights

Table 2: Correlation among environmental variables from PCA based on year-one data (values in bold indicate significance at P < 0.01)

	WaterT	DO (mg/l)	DO Sat %	pH	Salinity (ppt)	Turbdty (NTU)	SC (uS/cm)	TSS (mg/l)	Chl *a* (ug/l)	TN (mg/l)	Dis N (mg/l)	TP (mg/l)	DOC (mg/l)	Alk (mg/l)	Tot Si (mg/l)	Dis Si (mg/l)	TN: TP (in mole)
WaterT	1																
DO (mg/l)	**-0.89**	1															
DO Sat %	**-0.62**	**0.88**	1														
pH	0.05	0.02	0.12	1													
Salinity	-0.24	-0.02	-0.1	0.4	1												
Turbidity	0.17	-0.16	-0.17	0.25	0.13	1											
SC	-0.27	0.08	-0.08	0.4	**0.99**	0.13	1										
TSS	-0.2	0.07	-0.04	0.11	0.41	0.41	0.42	1									
Chl *a*	0.35	-0.16	-0.04	-0.02	-0.4	0.26	-0.4	-0.15	1								
TN	**0. 9**	-0.35	-0.18	-0.26	**-0.7**	0.1	**-0.76**	-0.36	0.53	1							
Dis N	0.27	-0.21	-0.18	-0.1	-0.16	0.01	-0.21	-0.09	0.01	0.25	1						
TP	**0.69**	-0.63	-0.47	0.14	0.09	**0. 6**	0.08	0.13	0.36	0.41	0.06	1					
DOC	0.42	-0.28	-0.19	-0.11	**-0.49**	0.1	-0.5	-0.17	0.34	**0. 8**	0.34	0.23	1				
Alk	-0.25	-0.07	-0.07	0.45	**0.98**	0.17	**0.97**	0.44	-0.36	**-0.73**	-0.19	0.1	-0.48	1			
Tot Si	0.35	-0.21	-0.12	-0.05	-0.31	0.41	-0.32	-0.05	0.45	0.52	0.25	0.4	0.46	-0.29	1		
Dis Si	0.4	-0.23	-0.12	-0.27	**-0.**	0.06	-0.51	-0.24	0.5	**0.61**	0.23	0.32	0.49	-0.48	**0.76**	1	
TN:TP	-0.38	0.43	0.27	-0.22	-0.47	-0.35	-0.45	-0.3	-0.13	0.13	0.07	-0.57	0.05	-0.49	-0.05	-0.01	1

Table 3: Correlation among environmental variables from PCA based on year-two data (values in bold indicate significance at P < 0.01)

	WaterT	DO_mg/l	DO_Sat%	pH	Salinity	Turbdty	SC_uS/cm	Secchi_f	TSS_mg/l	Chla	TN_mg/l	Dis_N	NO2+NO3	Dis_NH3	TP_ mg/l	Dis_P	DOC	TOC	Alk	Dis_ Si	TN:TP
WaterT	1.00																				
DO_mg/l	**-0.88**	1.00																			
DO_Sat%	-0.26	**0.60**	1.00																		
pH	-0.08	0.06	0.36	1.00																	
Salinity	-0.02	-0.13	0.16	**0.74**	1.00																
Turbdty	-0.08	0.11	0.15	0.10	0.18	1.00															
SC_uS/cm	-0.06	-0.09	0.18	**0.76**	**1.00**	0.19	1.00														
Secchi_f	-0.13	-0.05	-0.24	-0.10	-0.09	**-0.43**	-0.09	1.00													
TSS_mg/l	0.14	-0.11	0.07	0.27	0.38	**0.70**	0.37	**-0.52**	1.00												
Chla	0.10	0.01	-0.01	-0.01	-0.34	0.07	-0.33	-0.30	0.00	1.00											
TN_mg/l	0.26	-0.17	-0.24	**-0.59**	**-0.59**	0.22	**-0.60**	-0.25	-0.01	0.32	1.00										
Dis_N	0.19	-0.14	-0.24	**-0.74**	-0.54	0.26	**-0.56**	-0.09	-0.04	-0.05	**0.73**	1.00									
NO2+NO3	-0.24	0.31	-0.04	**-0.78**	**-0.67**	-0.04	**-0.68**	0.15	-0.26	-0.05	**0.51**	**0.68**	1.00								
Dis_NH3	0.05	-0.11	-0.05	0.01	0.21	0.30	0.20	-0.18	0.28	-0.27	0.14	0.29	0.04	1.00							
TP_mg/l	**0.45**	**-0.42**	-0.14	0.11	0.25	**0.49**	0.23	-0.61	**0.54**	0.09	**0.41**	0.20	-0.29	0.29	1.00						
Dis_P	**0.43**	**-0.52**	-0.30	-0.05	0.37	0.17	0.35	-0.24	0.27	-0.32	0.15	0.32	-0.16	**0.52**	**0.66**	1.00					
DOC	**0.49**	-0.28	-0.15	**-0.48**	-0.71	-0.10	**-0.72**	-0.17	-0.20	0.36	**0.61**	**0.50**	0.20	-0.10	0.19	-0.06	1.00				
TOC	**0.49**	-0.24	-0.03	-0.36	**-0.64**	-0.07	**-0.65**	-0.17	-0.21	0.34	**0.58**	0.35	0.18	-0.22	0.20	-0.15	**0.85**	1.00			
Alk	-0.06	-0.12	0.12	**0.75**	**0.96**	0.22	**0.97**	-0.12	**0.41**	-0.34	**-0.57**	**-0.53**	**-0.71**	0.19	0.31	**0.40**	**-0.68**	**-0.65**	1.00		
Dis_Si	**0.46**	-0.40	-0.37	**-0.51**	**-0.44**	-0.03	**-0.47**	-0.24	0.05	0.22	**0.54**	**0.52**	0.31	-0.02	0.36	0.27	**0.56**	**0.41**	**-0.42**	1.00	
TN:TP	-0.16	0.23	-0.08	**-0.75**	**-0.76**	-0.23	**-0.77**	0.31	-0.39	-0.03	0.36	**0.52**	**0.87**	-0.10	**-0.48**	-0.33	0.28	0.24	**-0.80**	0.36	1.00

Table 4: Summary of data sets for CCA analyses.

	Year-one	Year-two
Collection duration	August 2011-September 2012	October 2012-June 2013
Collection sites	8	6
# of samples	134	67
# of species	55	89
Environmental variables	14	11

Table 5: Explanatory power and the strength of the relationships between phytoplankton species composition and environmental variables, evaluated separately by the significance of the first CCA axis, based on Year-one data.

Environmental variable	Explains %	F_ratio	P
Salinity	7.8	11.2	0.002
TN_mg/l	6.2	8.7	0.002
Water temperature (^{o}C)	5.2	7.2	0.002
TN:TP (in mole)	4.4	6.1	0.002
Dissolved_Si	4	5.6	0.002
DOC_mg/l	3.7	5.1	0.002
DO_mg/l	3.6	4.9	0.002
TP_mg/l	3.5	4.7	0.002
TSS mg/l	3.3	4.5	0.002
Total Si	3.1	4.3	0.002
Chla_ug/l	2.5	3.4	0.002
Turbidity (NTU)	2.4	3.3	0.002
pH	2.2	2.9	0.002
Dis_N_mg/l	0.8	1	0.376

Table 6: Explanatory power and the strength of the relationships between phytoplankton species composition and environmental variables, evaluated separately by the significance of the first CCA axis, based on Year-two data.

Environmental variable	Explains %	F_ratio	P
Water temperature (^{o}C)	8.6	6.1	0.002
DO_mg/l	7.3	5.1	0.002
Dissolved Si_mg/l	4.8	3.3	0.002
TOC mg/l	4.6	3.2	0.002
TN_mg/l	4.4	3	0.002
TP_mg/l	4.1	2.8	0.002
TSS mg/l	3.2	2.2	0.004
Salinity (ppt)	3.1	2.1	0.002
TN:TP (in mole)	2.7	1.8	0.01
Chlorophyll *a* (ug/l)	2.6	1.7	0.016
Dis NH3_mg/l	2.2	1.5	0.046

LIST OF FIGURES

Figures

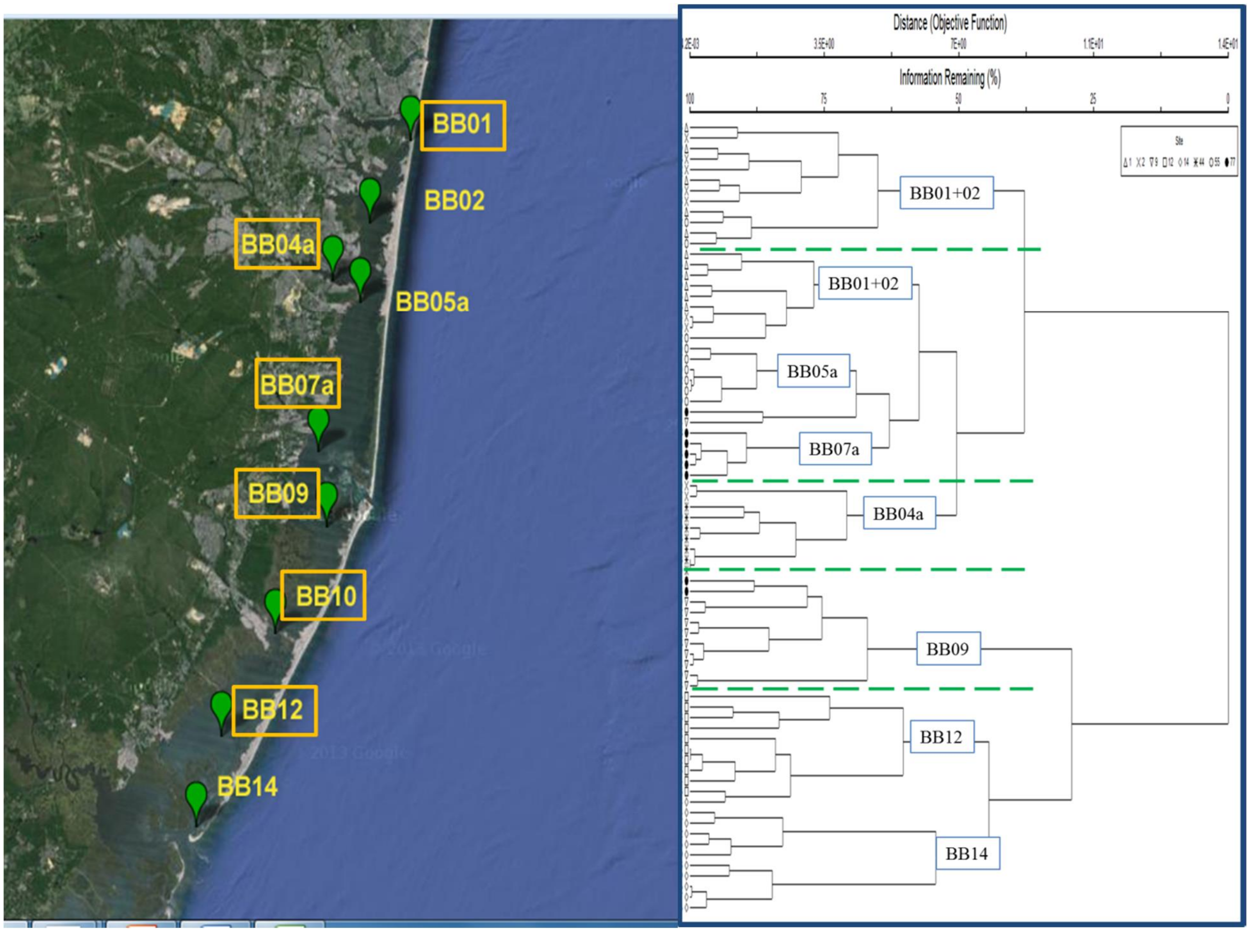


Fig. 1. **Left panel**: Map of sites for phytoplankton sample collection 2011-13. Samples from six sites (framed) were collected from 2012-2013. Note BB04a, BB05a and BB07a were shifted from BB04, BB05, BB07 (not shown) after May 2012. **Right panel**: Cluster analysis of sites based on 2011-12 data (8 sites with exclusion of BB10).

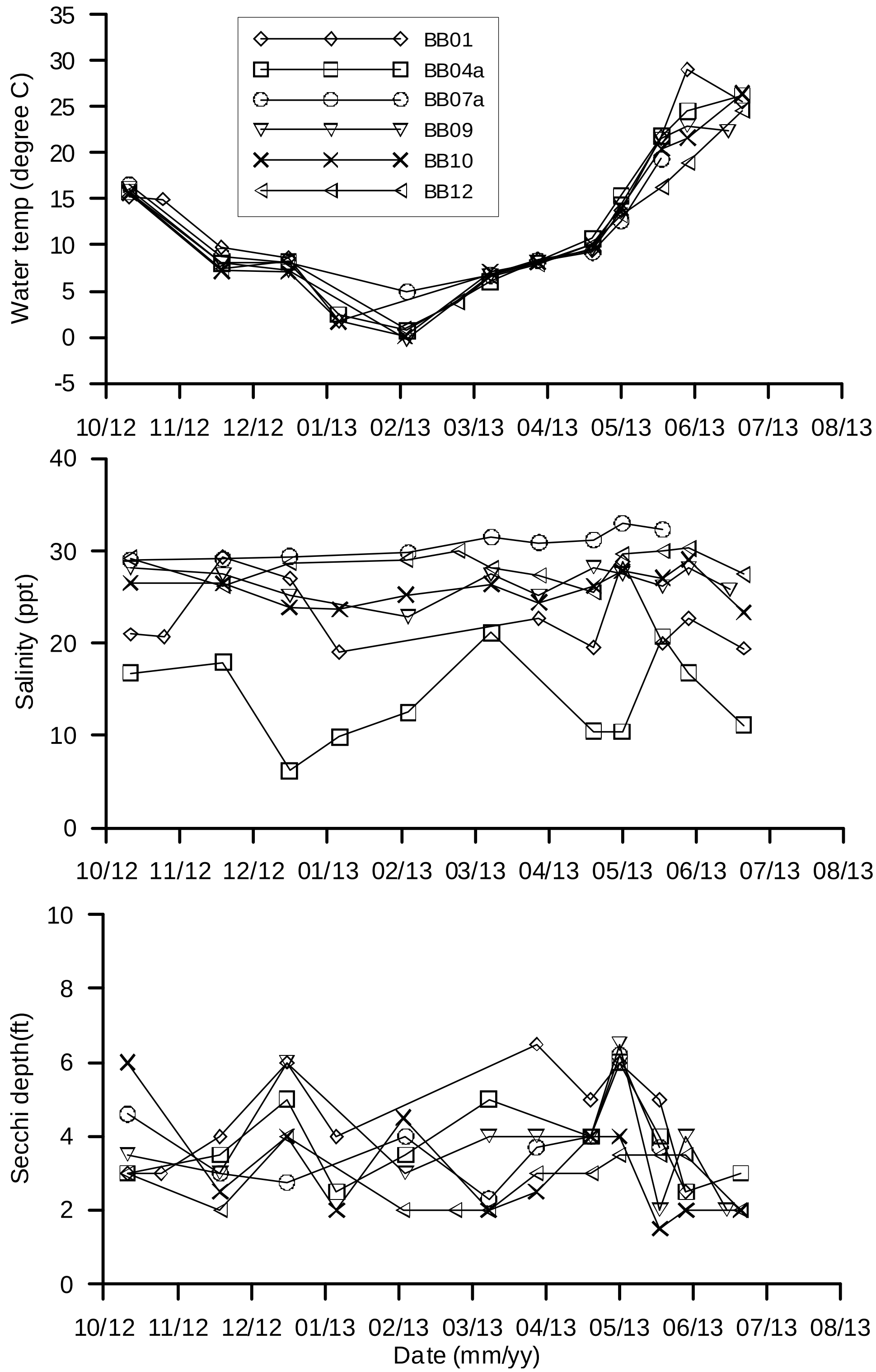


Fig. 2. Change of water temperature, salinity and Secchi depth at phytoplankton collection sites in BB-LEH from October 2012 to August 2013. Data from NJDEP water quality monitoring, http://www.nj.gov/dep/barnegatbay/bbmapviewer.htm.

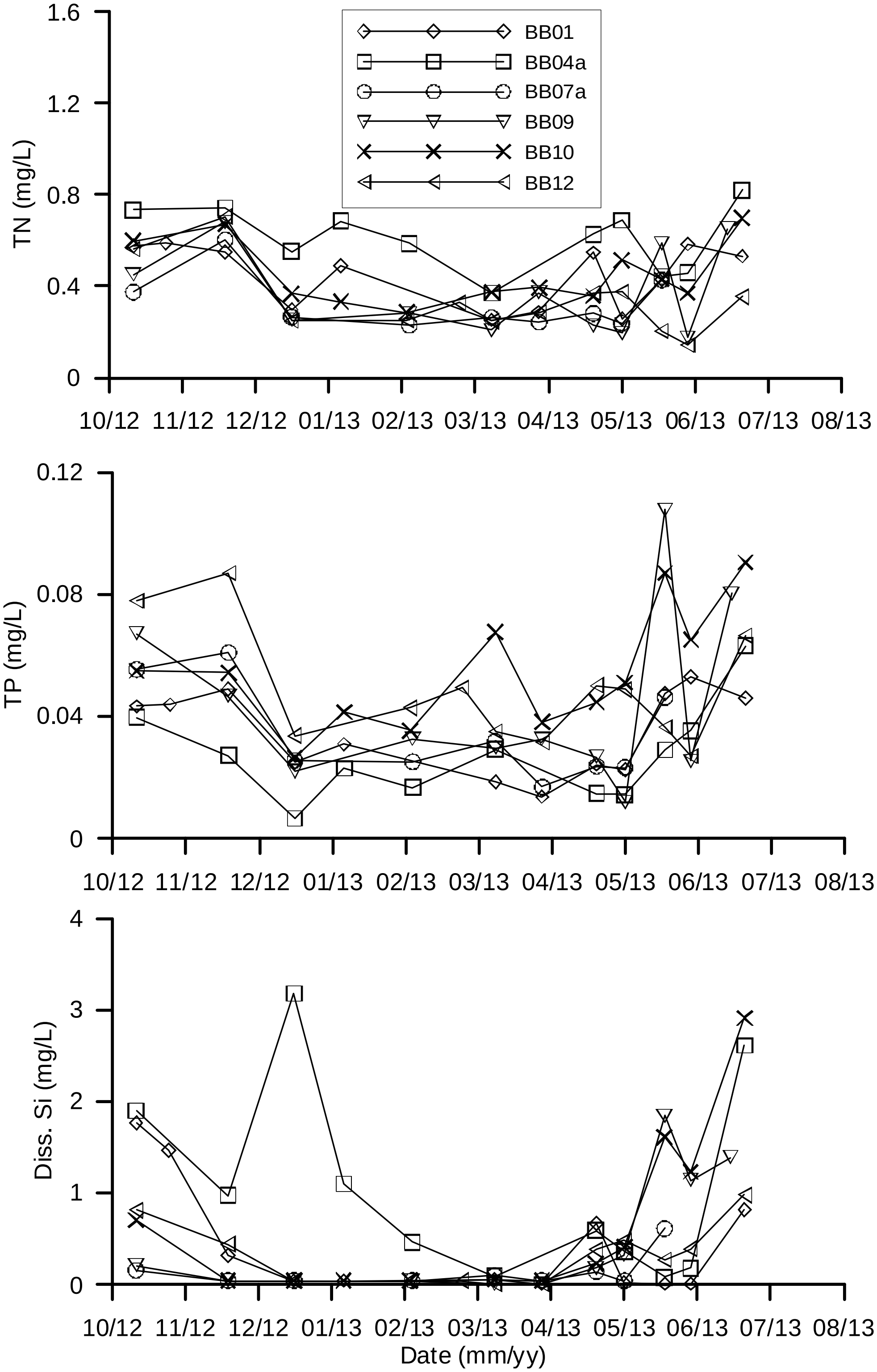


Fig. 3. Change of total nitrogen (TN), total phosphorus (TP) and dissolved Si (DSi) at phytoplankton collection sites in BB-LEH from August 2011 to September 2012. Data from NJDEP water quality monitoring: http://www.nj.gov/dep/barnegatbay/bbmapviewer.htm.

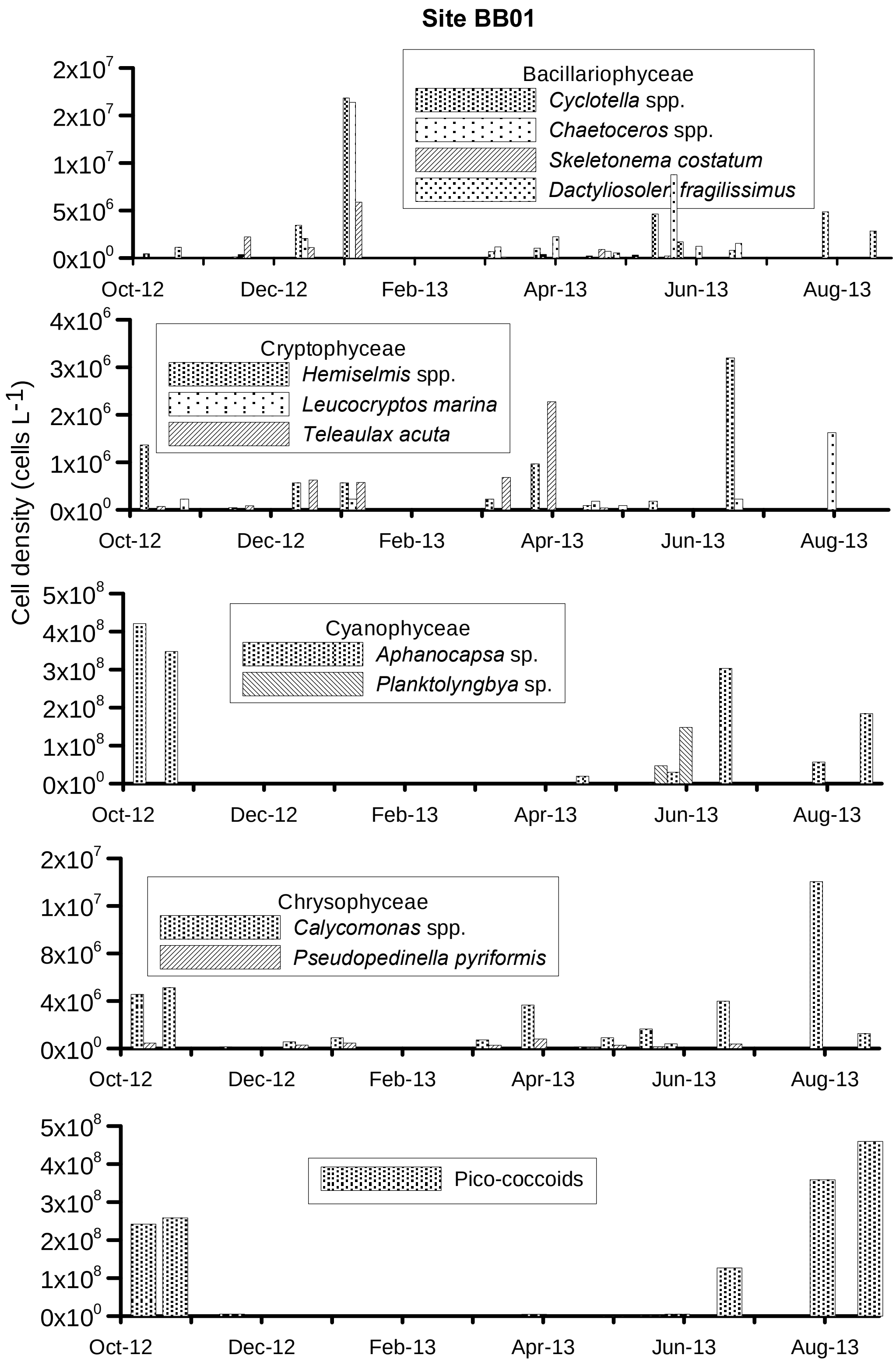


Fig. 4. Abundance and seasonal changes of some dominant species at site BB01 from October 2012 to August 2013.

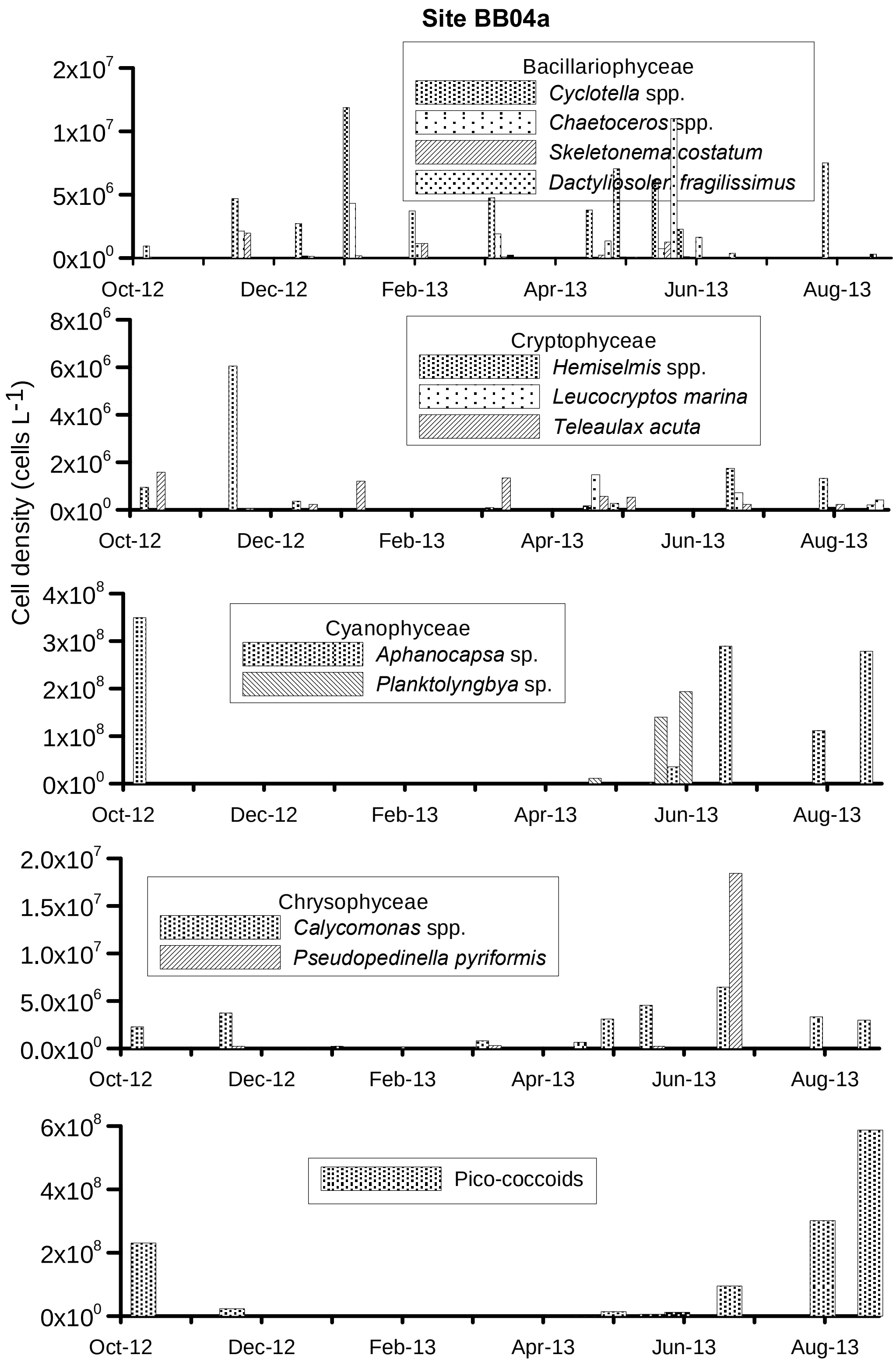


Fig. 5. Abundance and seasonal changes of some dominant species at site BB04a from October 2012 to August 2013.

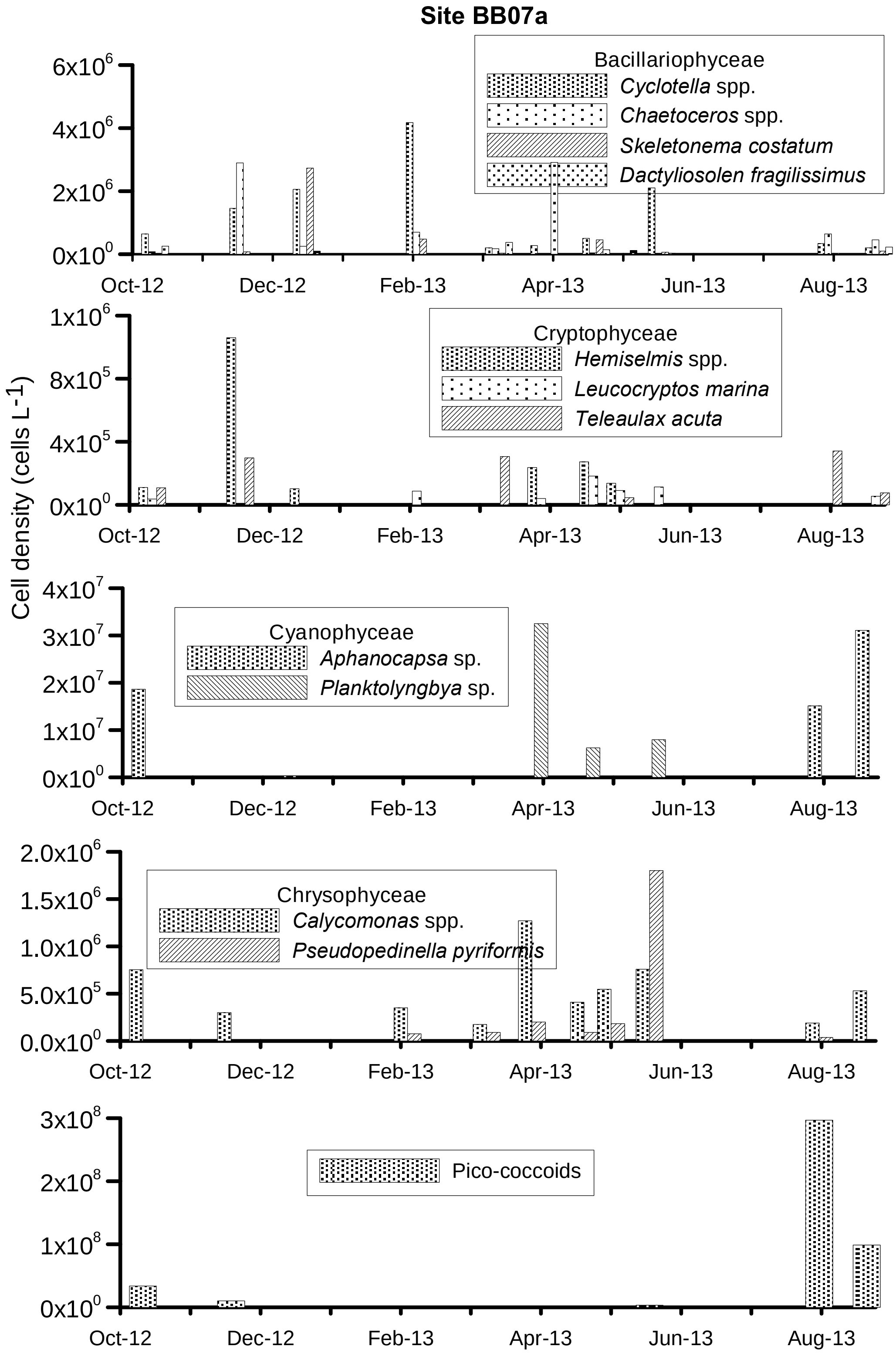


Fig. 6. Abundance and seasonal changes of some dominant species at site BB07a from October 2012 to August 2013.

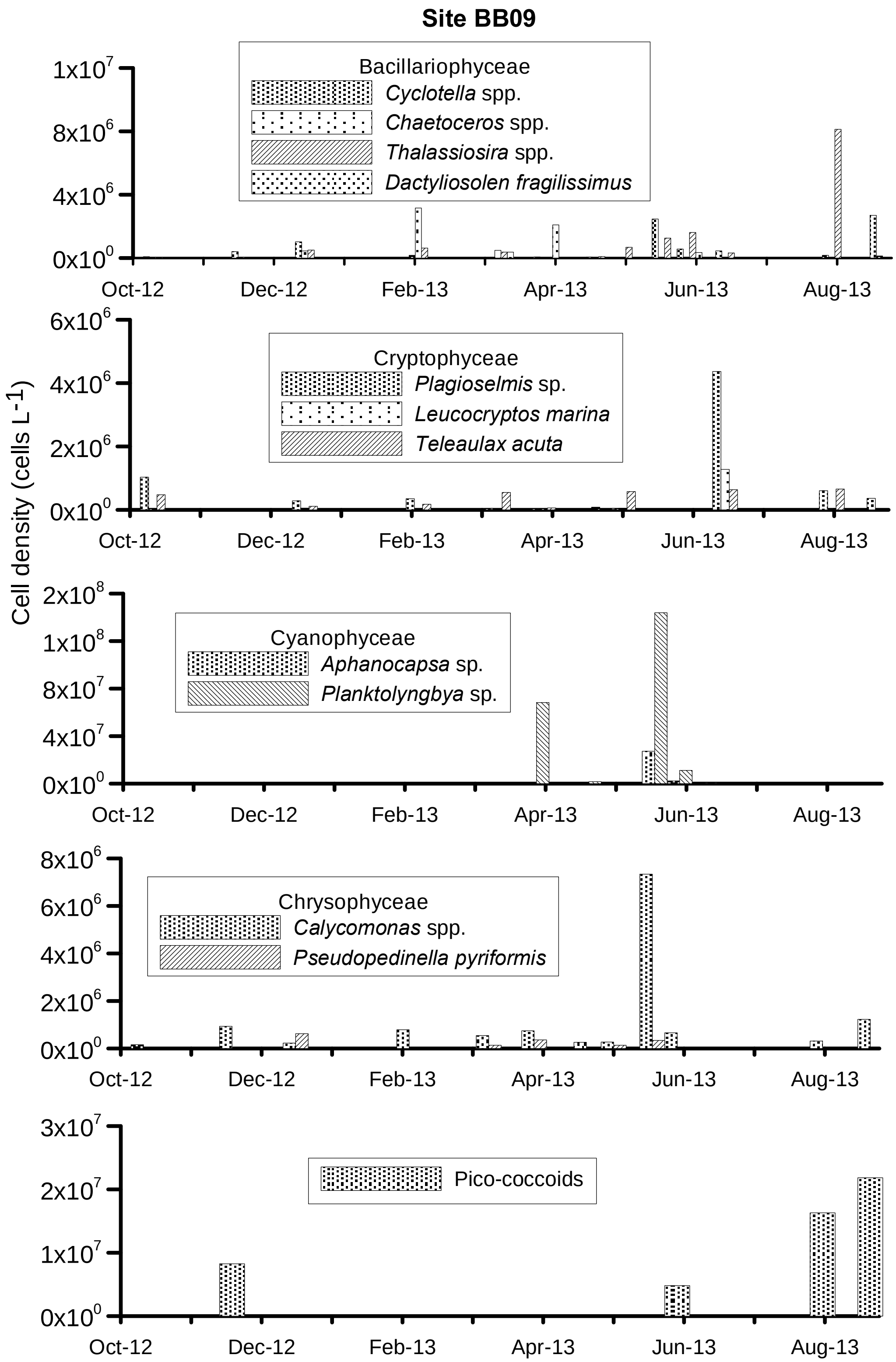


Fig. 7. Abundance and seasonal changes of some dominant species at site BB09 from October 2012 to August 2013.

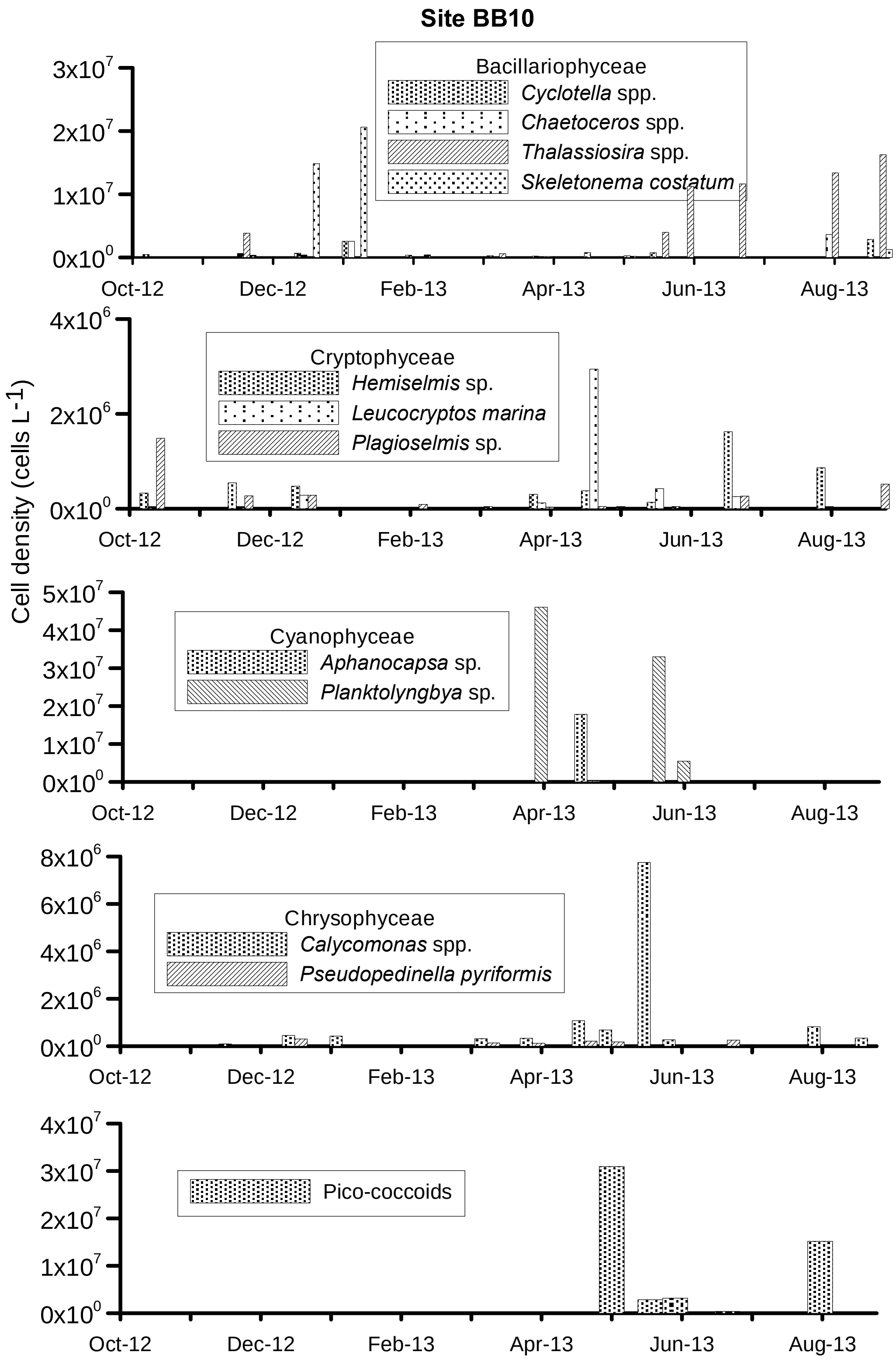


Fig. 8. Abundance and seasonal changes of some dominant species at site BB10 from October 2012 to August 2013.

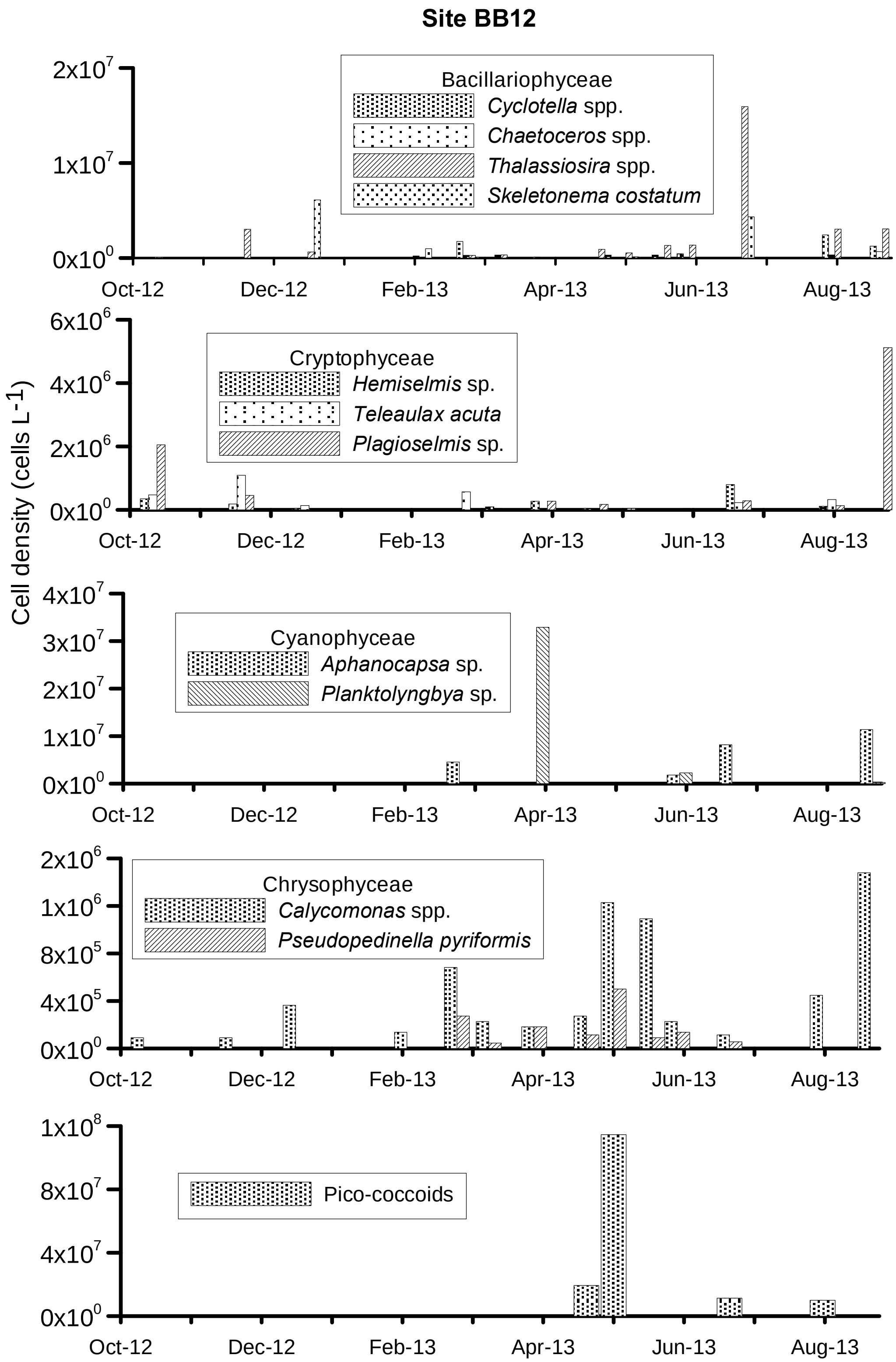


Fig. 9. Abundance and seasonal changes of some dominant species at site BB12 from October 2012 to August 2013.

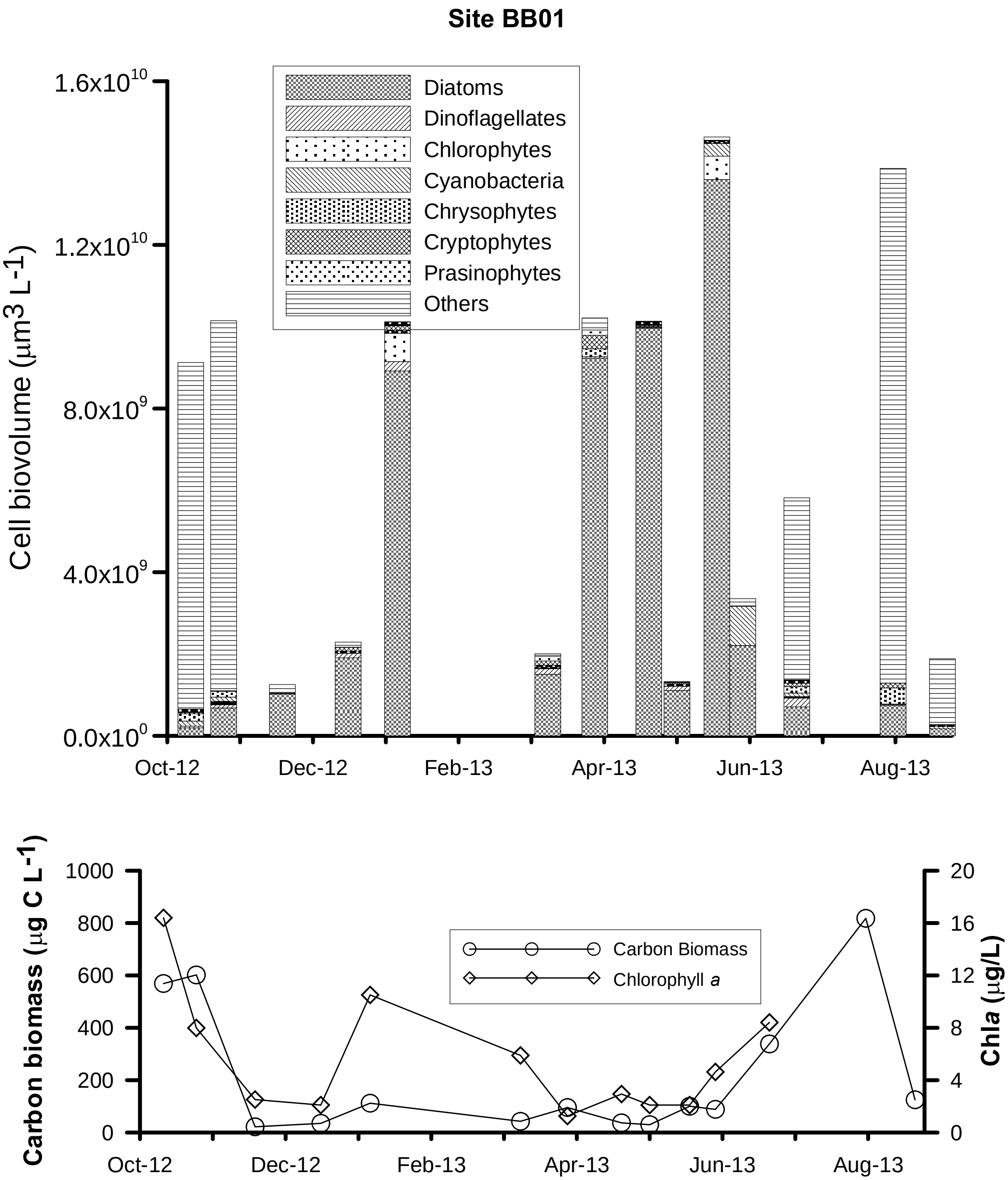


Fig. 10. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB01 from October 2012 to August 2013.

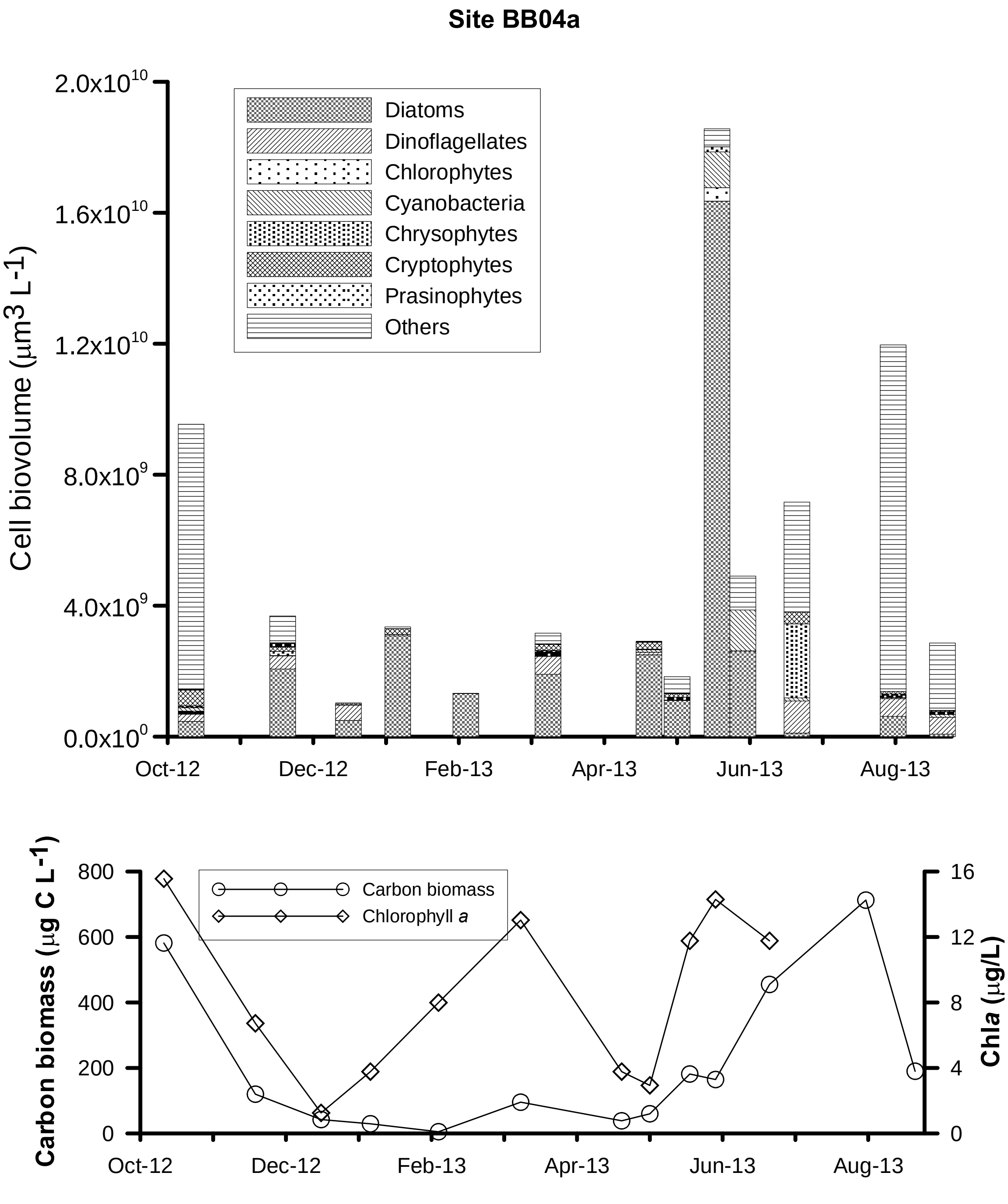


Fig. 11. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB04a from October 2012 to August 2013.

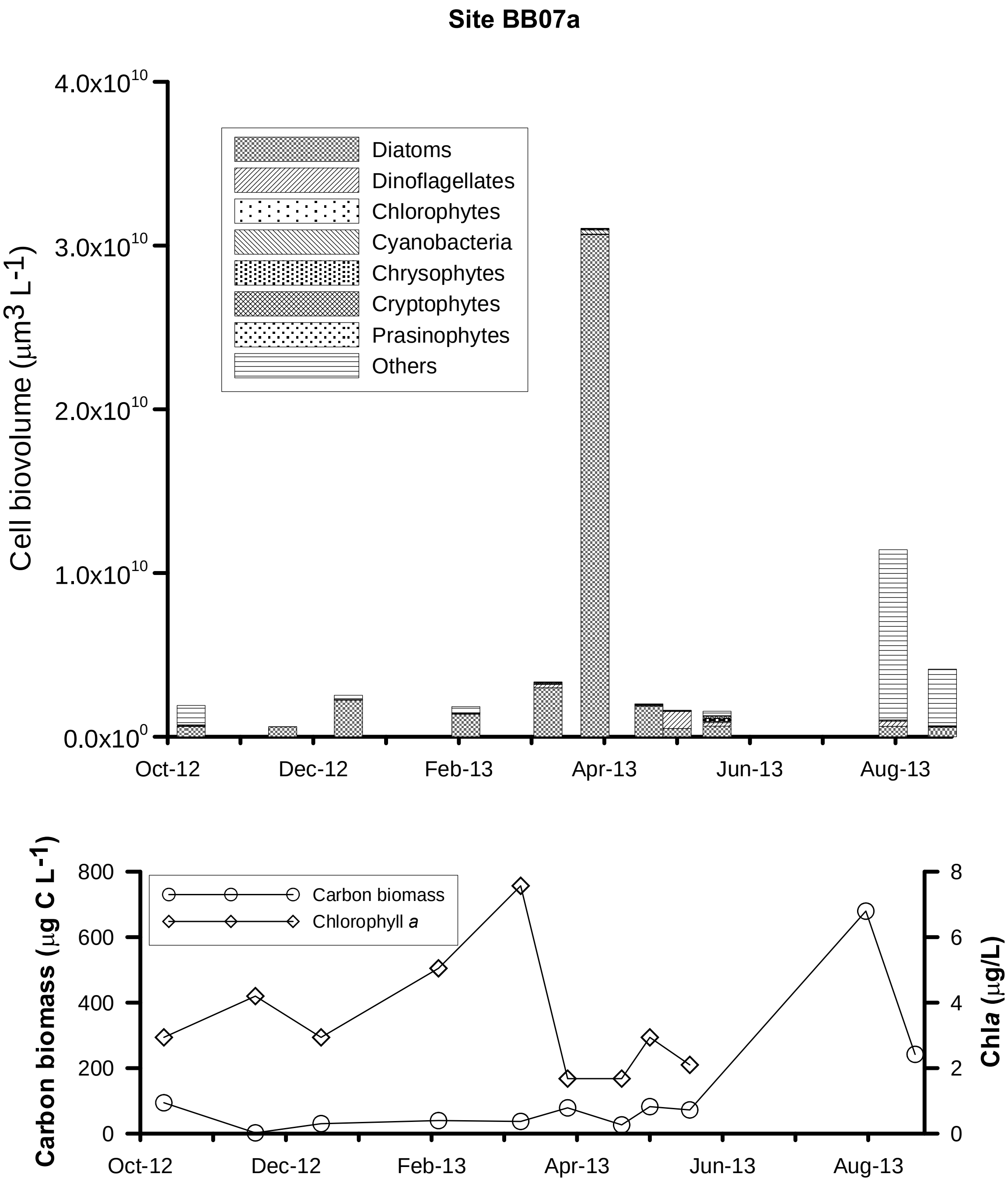


Fig. 12. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB07a from October 2012 to August 2013.

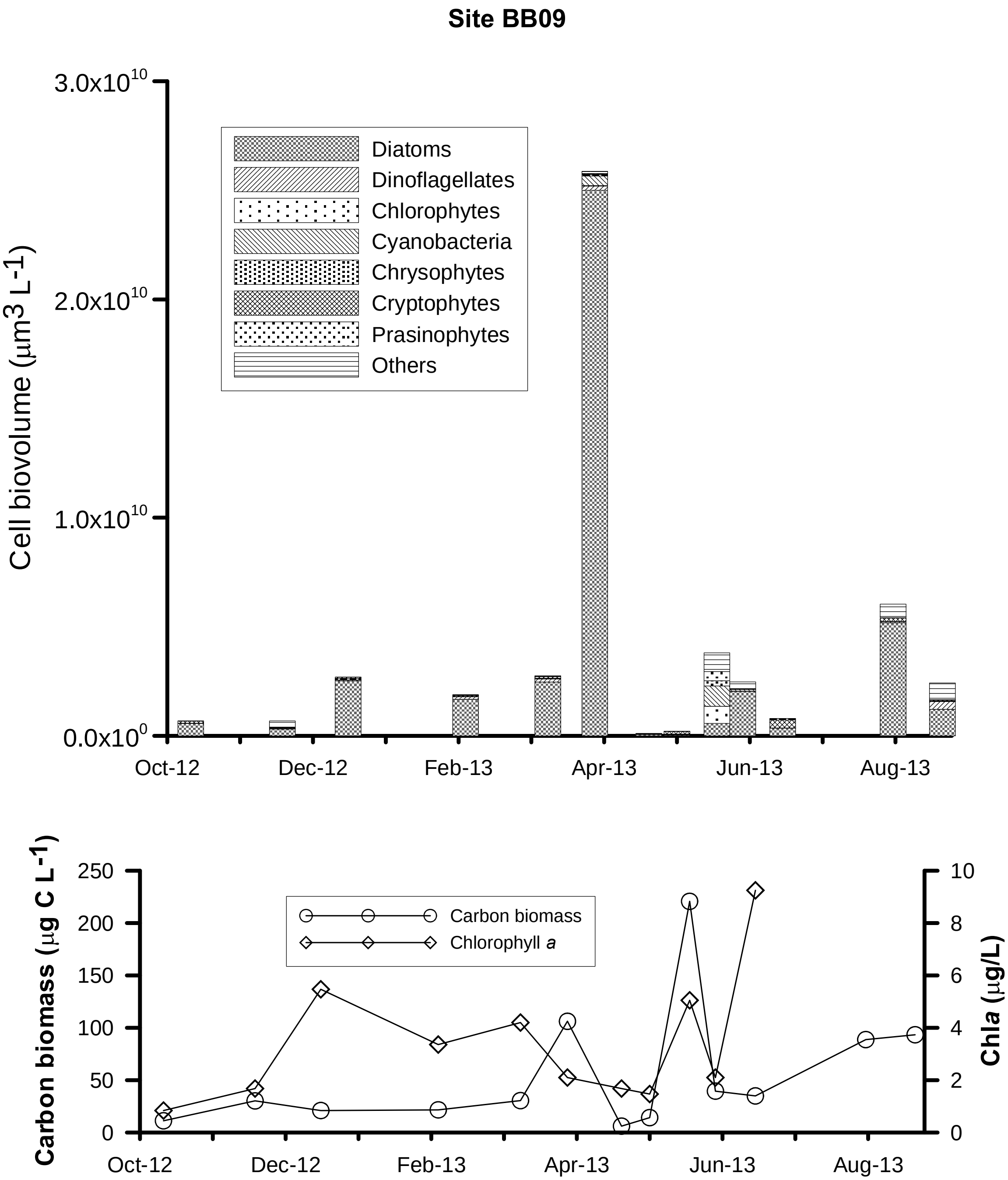


Fig. 13. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB01 from October 2012 to August 2013.

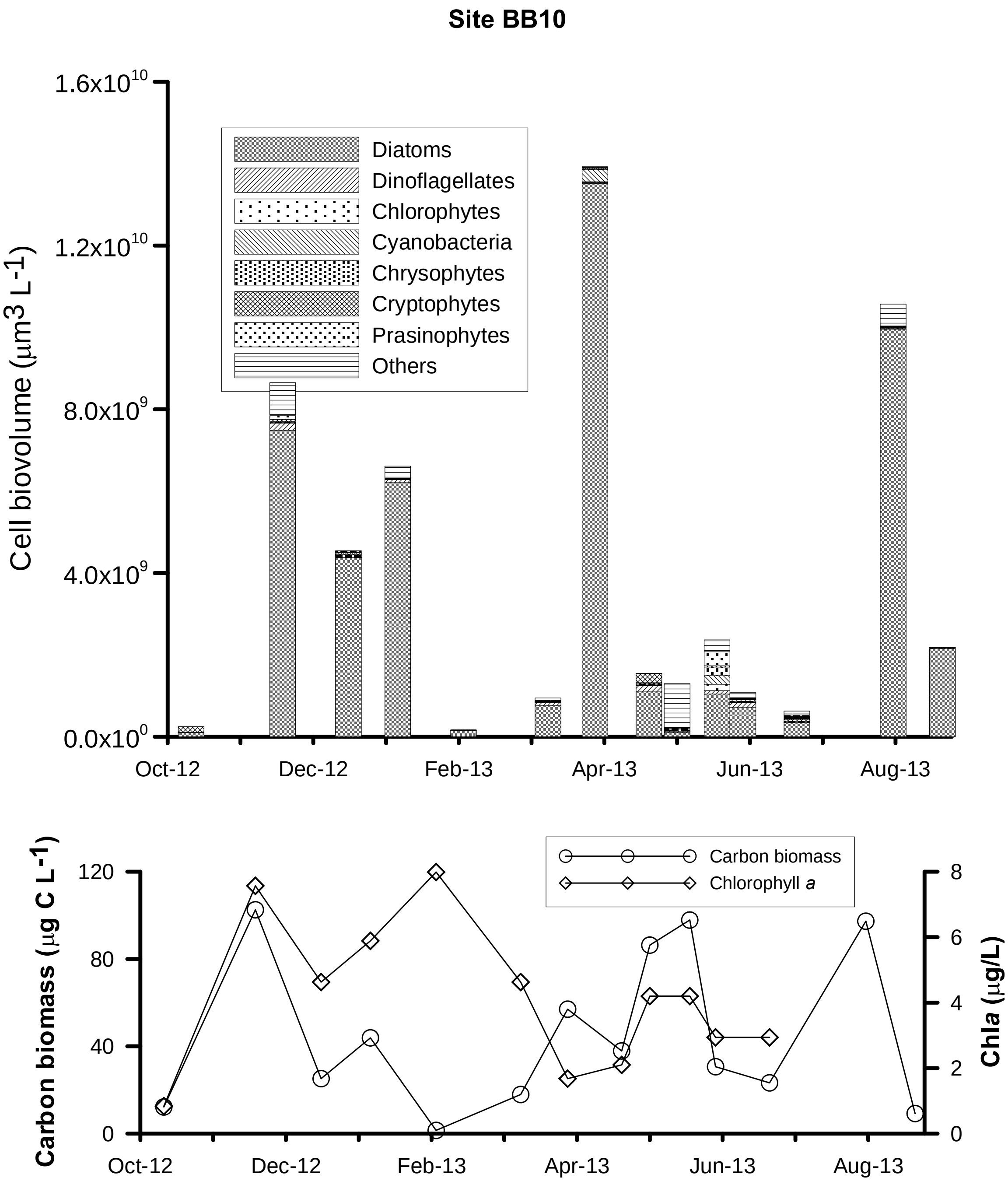


Fig. 14. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB10 from October 2012 to August 2013.

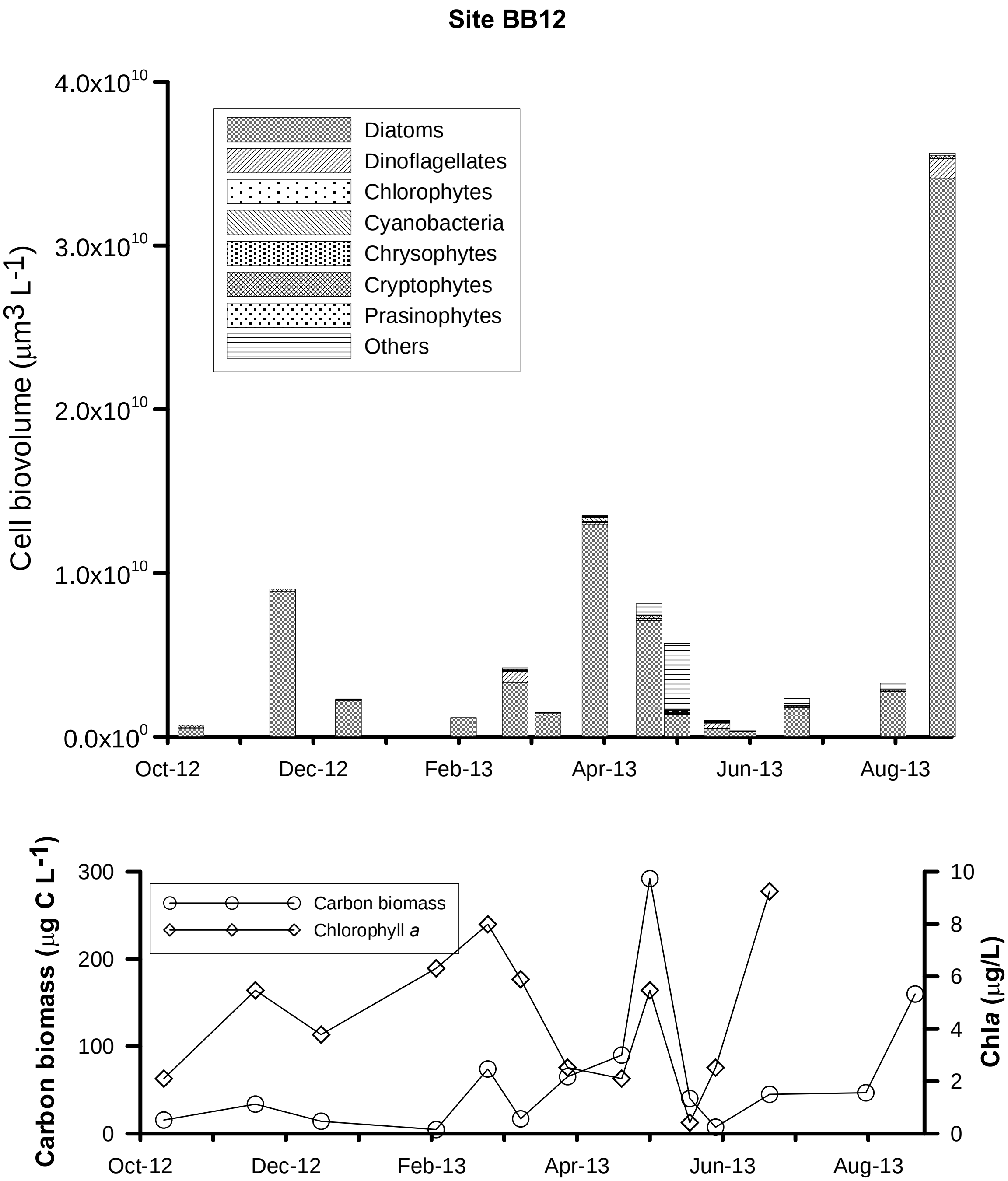


Fig. 15. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB12 from October 2012 to August 2013.

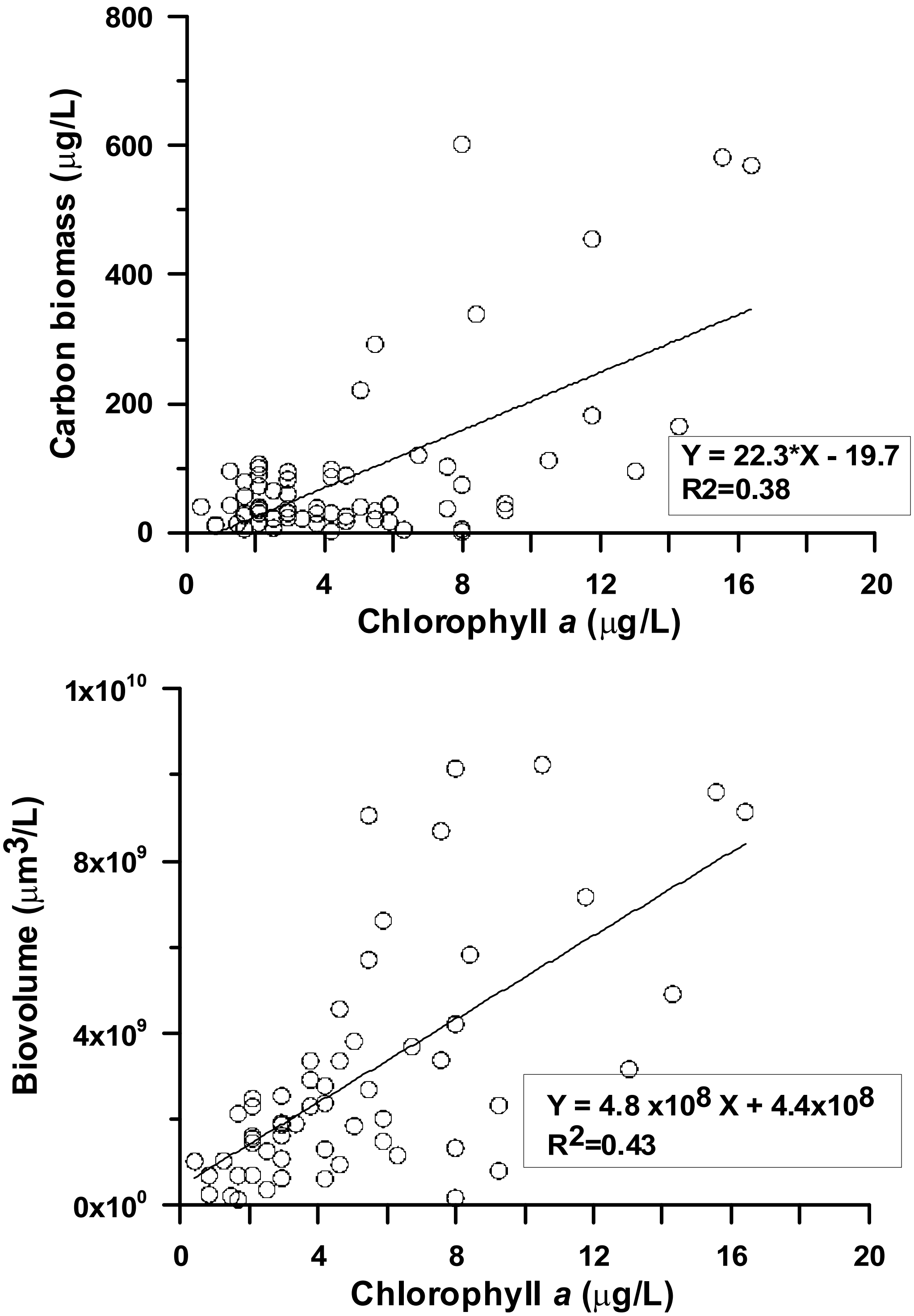


Fig. 16. Correlation of chlorophyll *a* and biovolume, and estimated carbon biomass based on phytoplankton community data from October 2012 to August 2013.

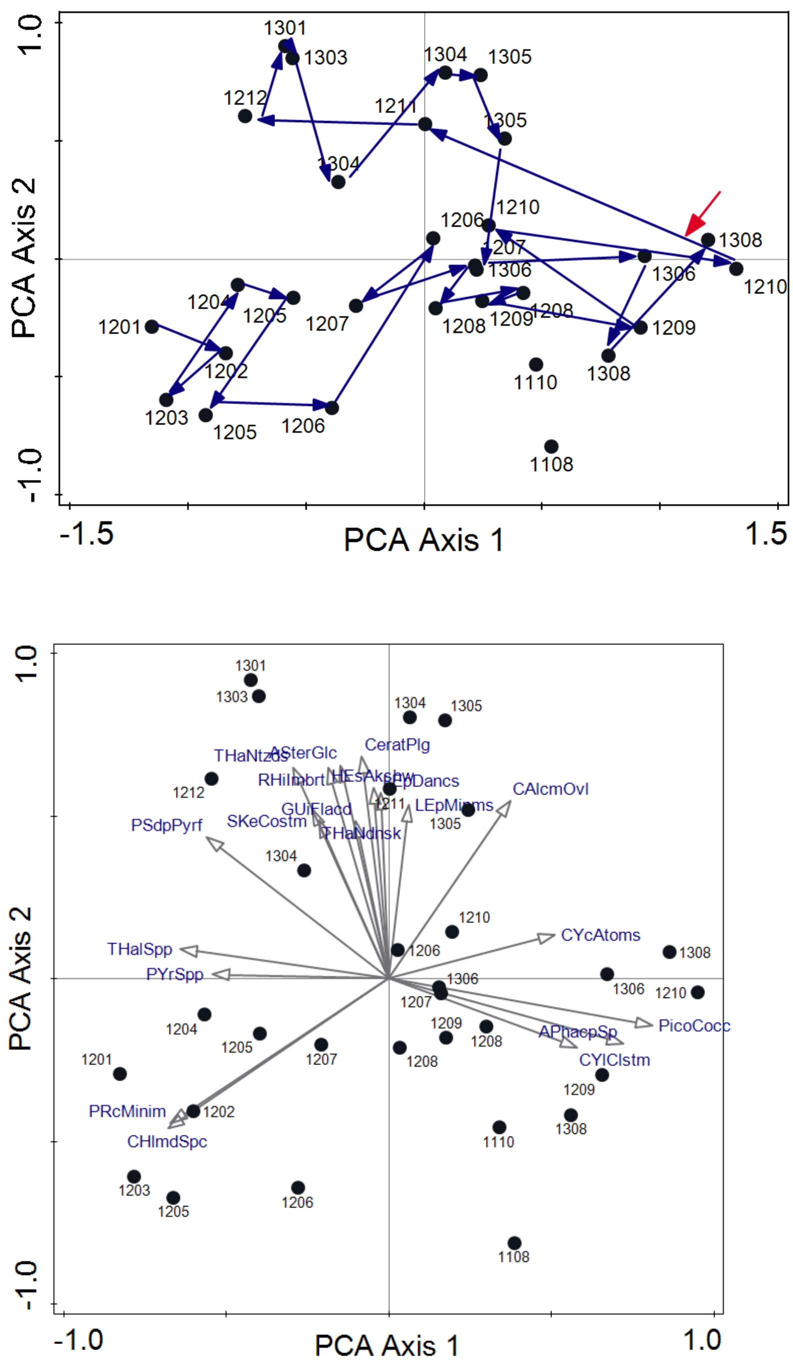
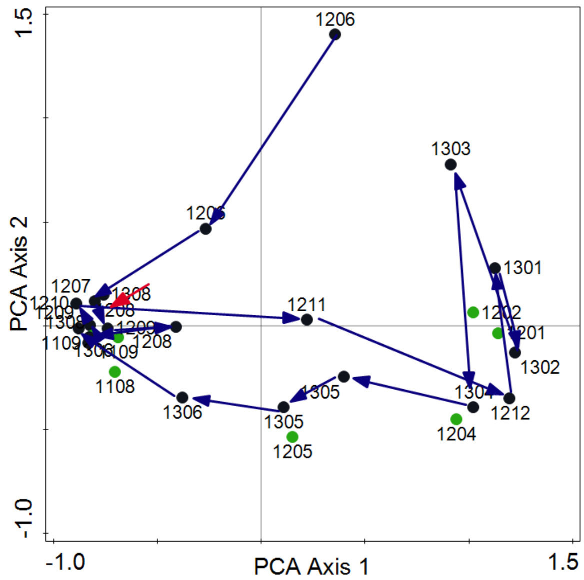


Fig. 17. Year-to-year changes of phytoplankton community at BB01 from August 2011 to August 2013. Each dot in the diagrams represents phytoplankton community in one sample. Samples labelled as collection year and month (YYMM). **Upper panel**: sample scatter diagram: the relative distance between samples reflects relative similarity in species composition; **Lower panel**: sample-species biplot, species arrows point to the direction of steepest increase of species values (see more detailed in Methods).



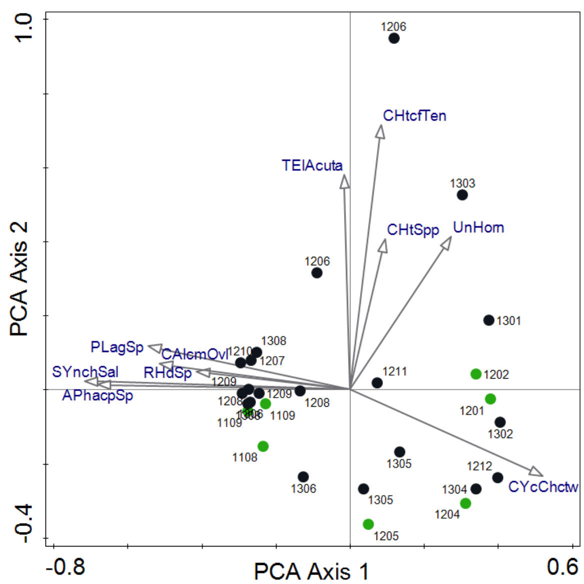


Fig. 18. Year-to-year changes of phytoplankton community at BB04a from August 2011 to August 2013. Each dot in the diagrams represents phytoplankton community in one sample. Samples labelled as collection year and month (YYMM). Upper panel: sample scatter diagram, the relative distance between samples reflects relative similarity in species composition; Lower panel: sample-species biplot, species arrows point to the direction of steepest increase of species values (see more detailed in Methods).

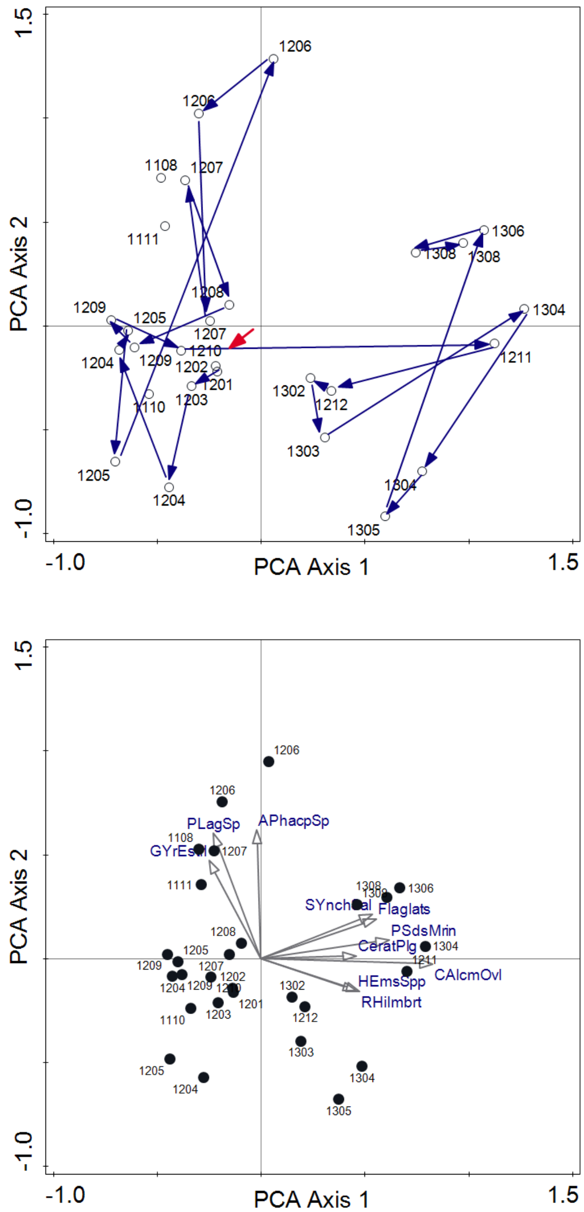


Fig. 19. Year-to-year changes of phytoplankton community at BB09 from August 2011 to August 2013. Each dot in the diagrams represents phytoplankton community in one sample. Samples labelled as collection year and month (YYMM). **Upper panel**: sample scatter diagram: the relative distance between samples reflects relative similarity in species composition; **Lower panel**: sample-species biplot, species arrows point to the direction of steepest increase of species values (see more detailed in Methods).

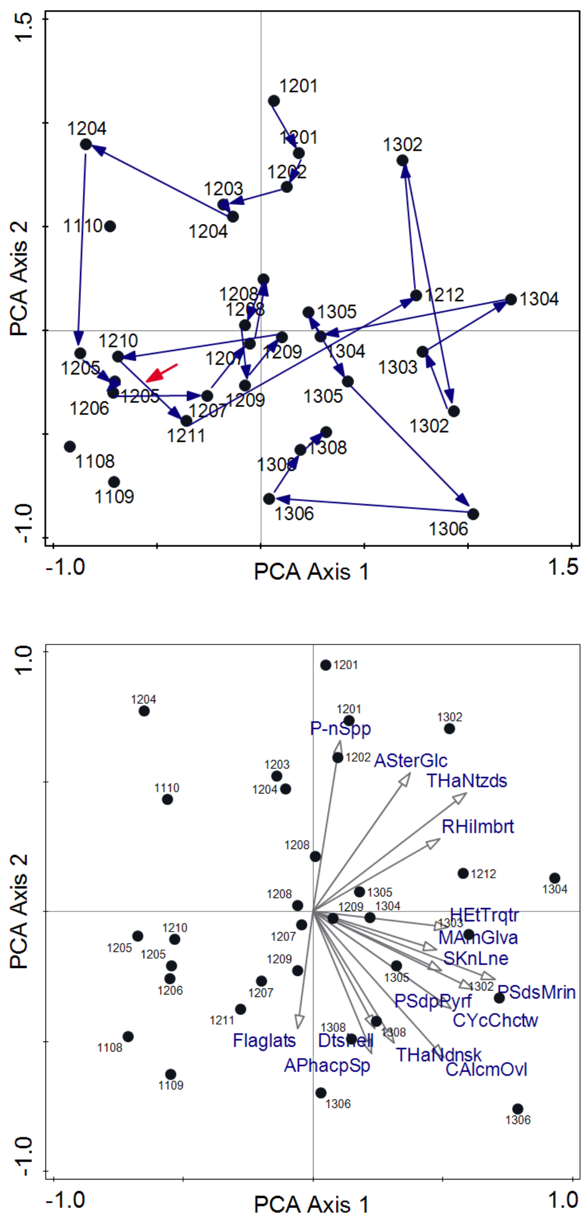


Fig. 20. Year-to-year changes of phytoplankton community at BB12 from August 2011 to August 2013. Each dot in the diagrams represents phytoplankton community in one sample. Samples labelled as collection year and month (YYMM). **Upper panel**: sample scatter diagram: the relative distance between samples reflects relative similarity in species composition; **Lower panel**: sample-species biplot, species arrows point to the direction of steepest increase of species values (see more detailed in Methods).

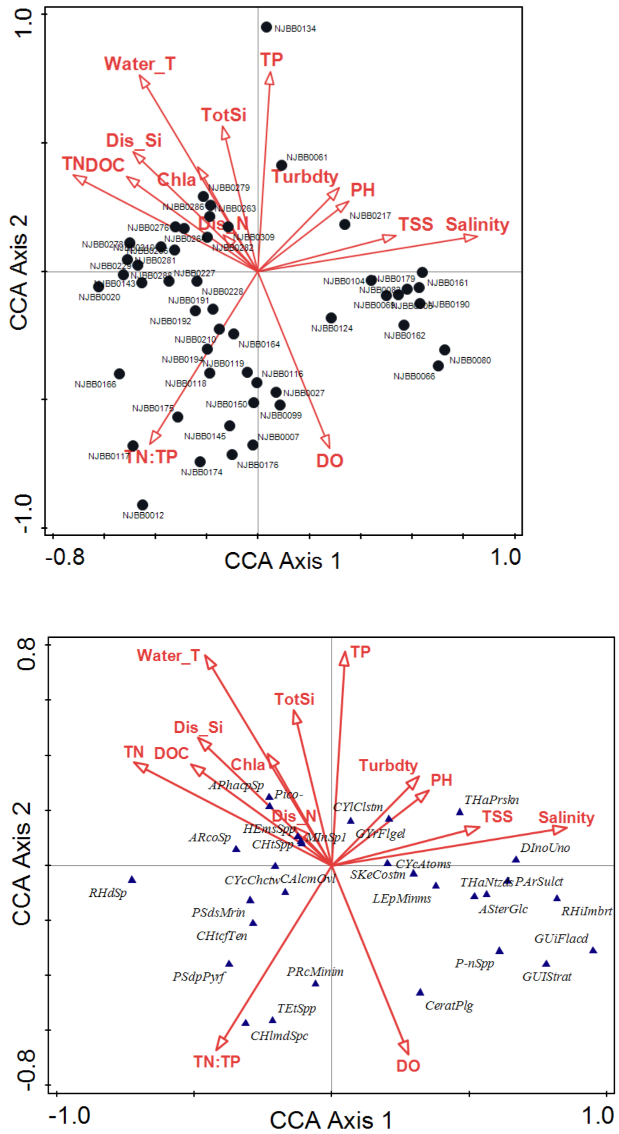


Fig. 21. Results of canonical correspondence analysis (CCA) based on year-one phytoplankton data collected from August 2011 to September 2012. **Upper panel**: samples-environmental variables biplot showing the changes of the phytoplankton community of 134 samples in environmental conditions; **Lower panel**: species-environmental variables biplot showing changes in species composition explained by environmental conditions. The first 30 species with highest weight are shown.

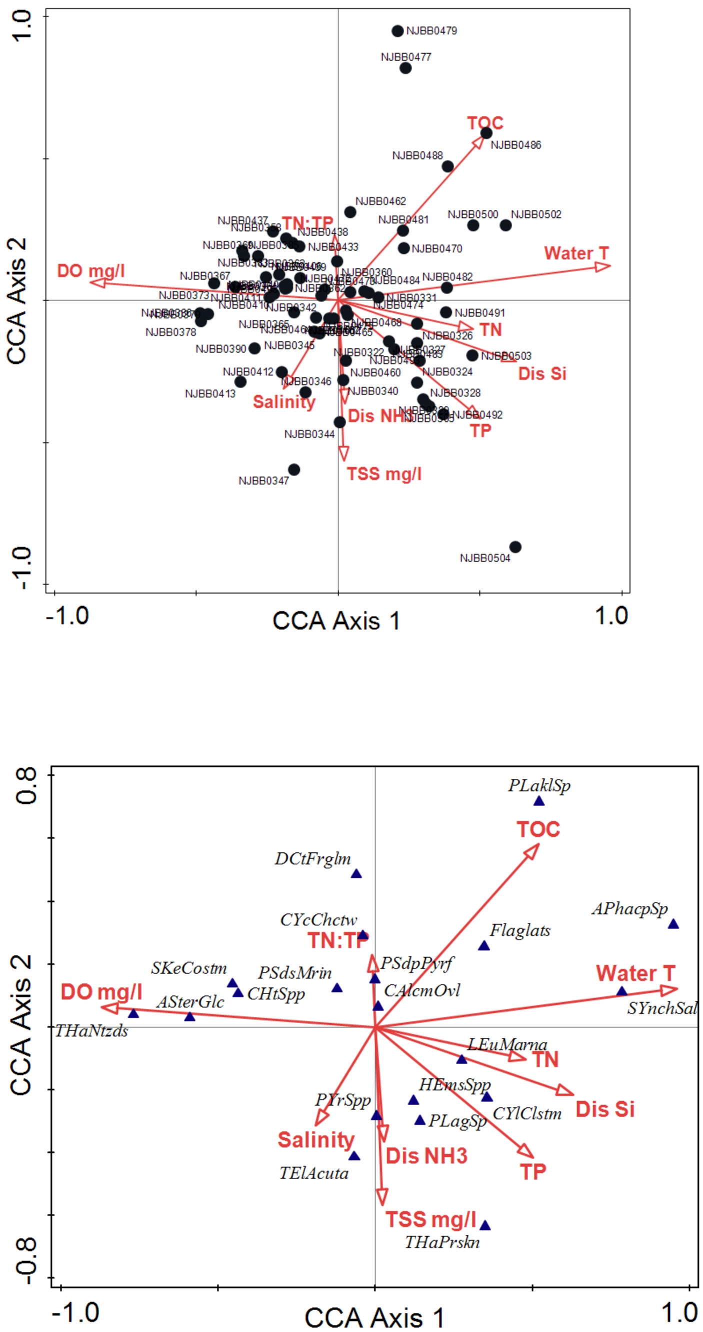


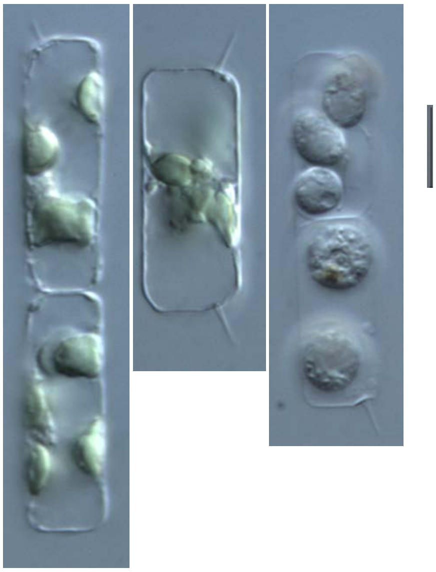
Fig. 22. Results of canonical correspondence analysis (CCA) based on year-two phytoplankton data collected from October 2012 to August 2013 at six sites. **Upper panel**: samples-environmental variables biplot showing the changes of the phytoplankton community of 67 samples in environmental conditions; **Lower panel**: species-environmental variables biplot showing changes in species composition explained by environmental conditions. The first 20 species with highest weight are shown.

APPENDICES

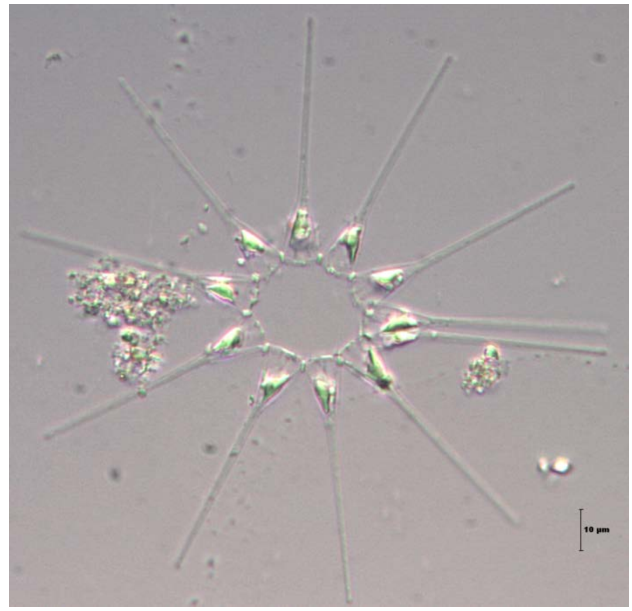
Appendix 1(CD): Plates: Image documentation on some abundant phytoplankton species.

Appendix 2 (CD): Excel files with data on phytoplankton species cell density, biovolume calculation and carbon estimation for sites BB01, BB04a, BB07a, BB09, BB10, and BB12 from October 2012 to August 2013.

Plate 1   Diatoms



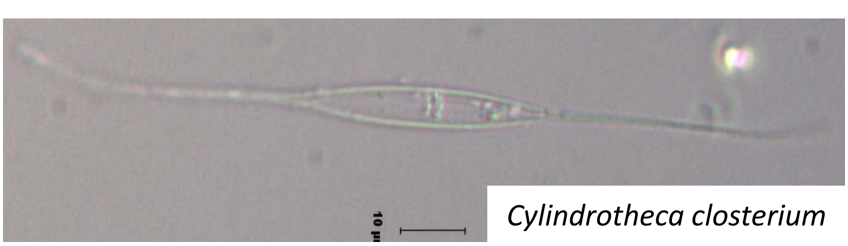
Dactyliosolen fragilissimus



Asterionellopsis glacialis

Cyclotella choctawatcheena

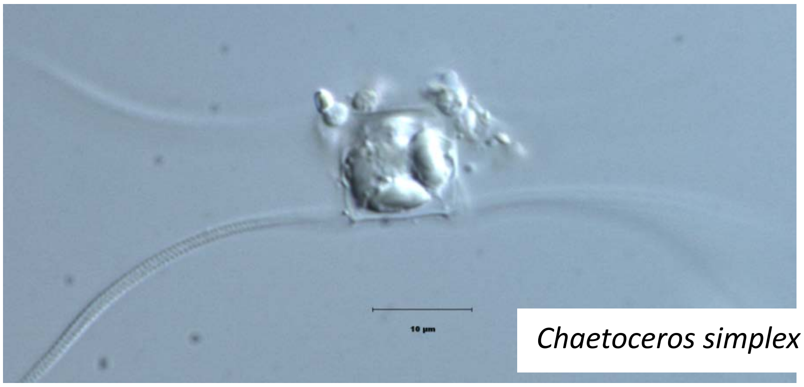
Cyclotella atomus



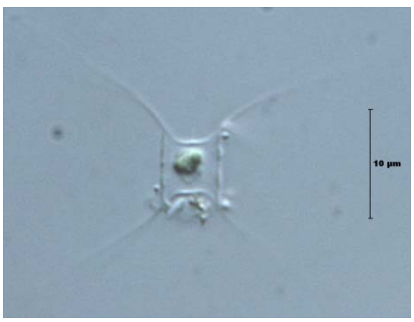
Cylindrotheca closterium

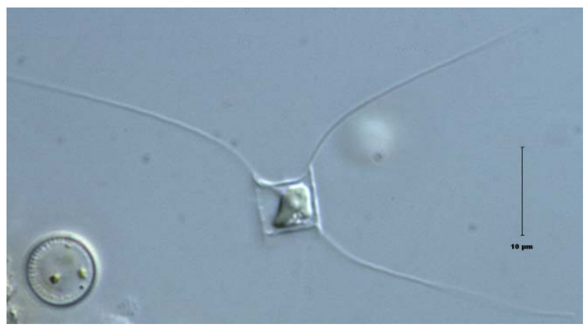
Plate 2 Diatoms

Chaetoceros spp.



Chaetoceros simplex

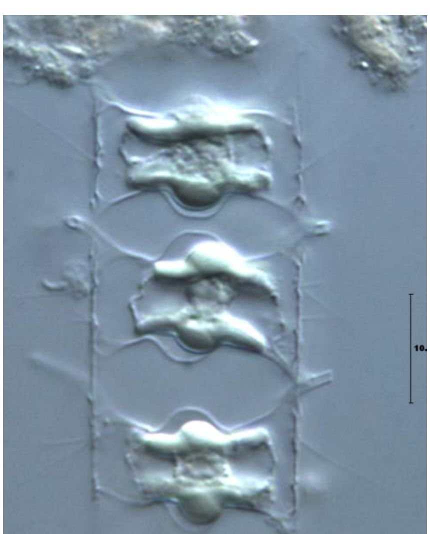




Chaetoceros tenuissimus

Ch. Subtilis var. abnormis fo. simplex

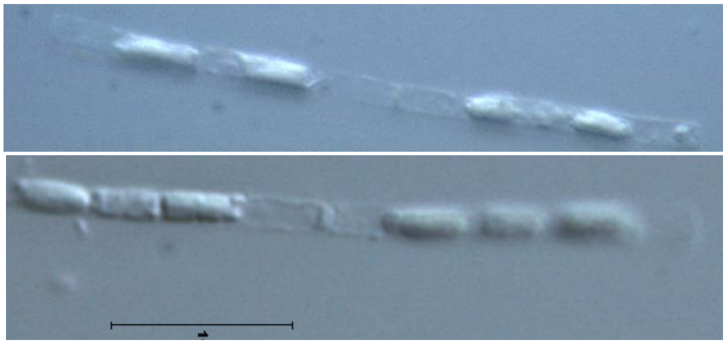
Ch. danics



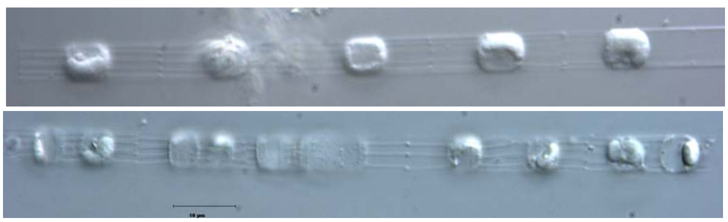
Ch. didymus

Attheya decora

Plate 3   Diatoms

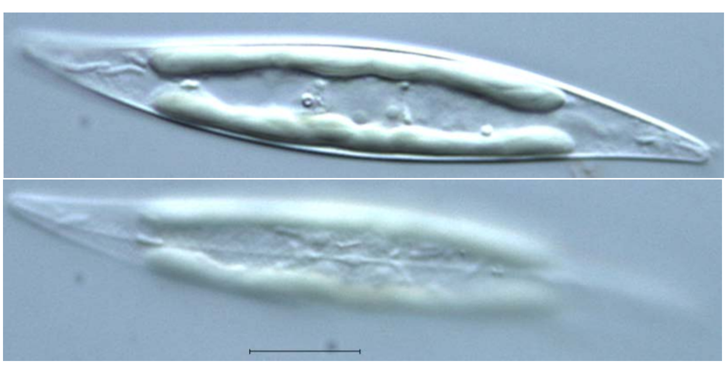


Leptocylindrus minimus



Skeletonema "costatum'

Pseudo-nitzschia pungens

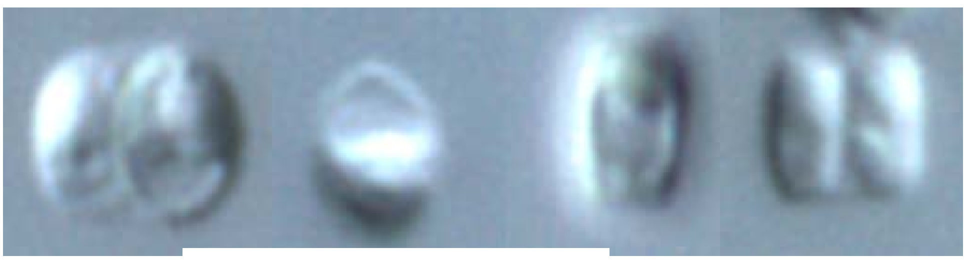


Pleurosigma salinarum

Rhizosolenia sp.

Plate 4 Diatoms

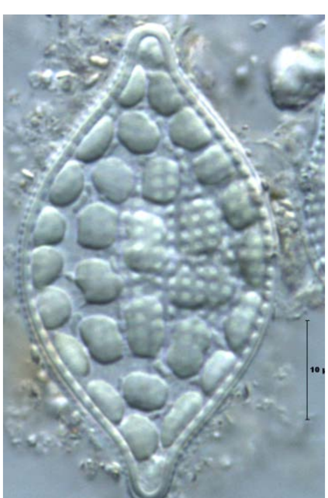
Minidiscus spp.



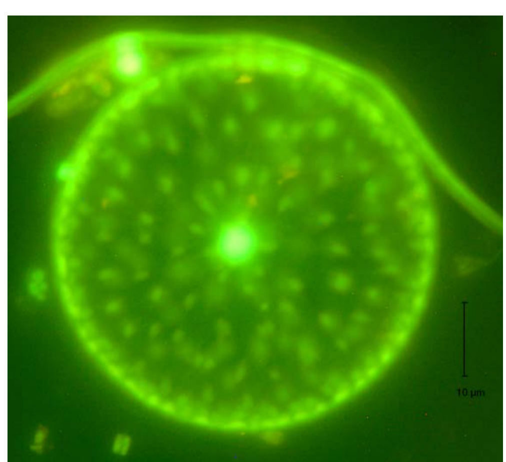
PhaeodactylumQ tricornutum

Minutocellus scriptus

Thalassionema nitzschioides



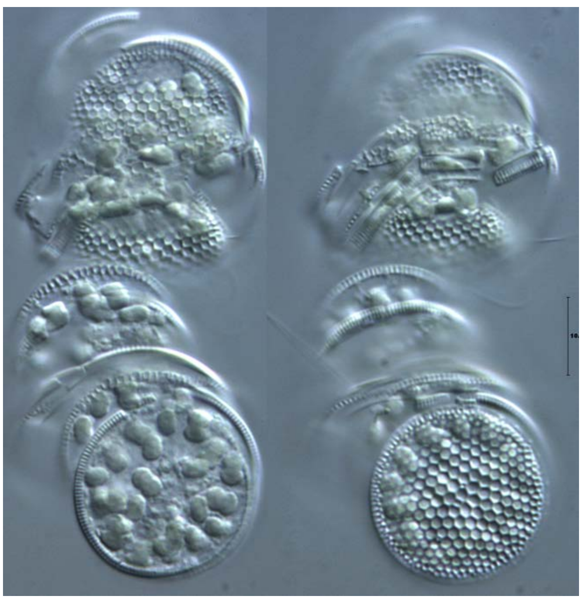
Rhaphoneis amphiceros



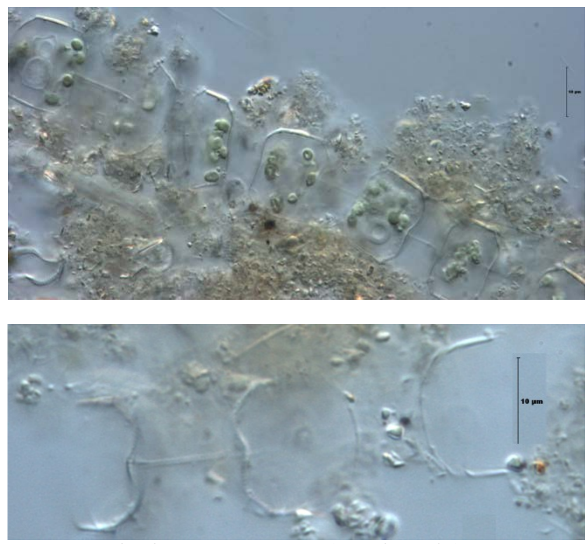
Coscinodiscus concinnus

Plate 5 Diatoms

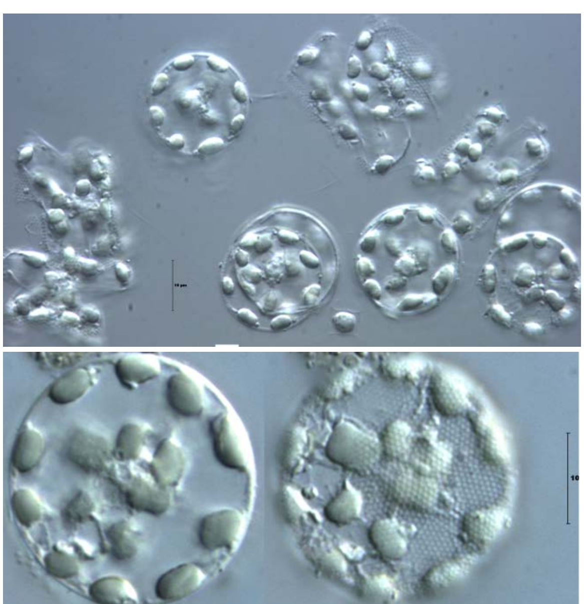
Thalassiosira proschkinae



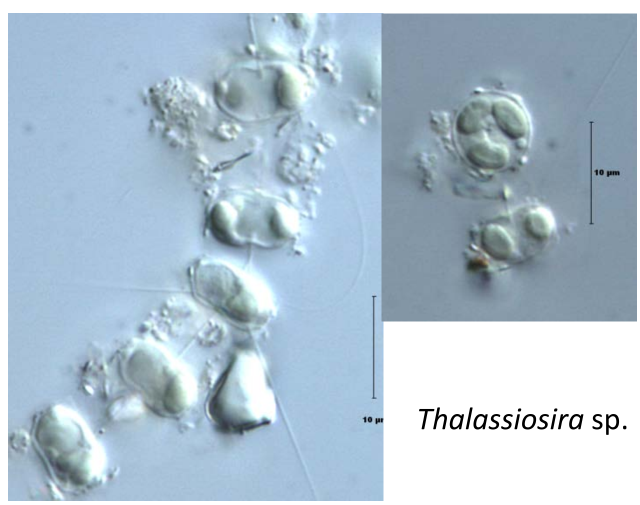
Thalassiosira oestrupii



Thalassiosira nordenskioeldii

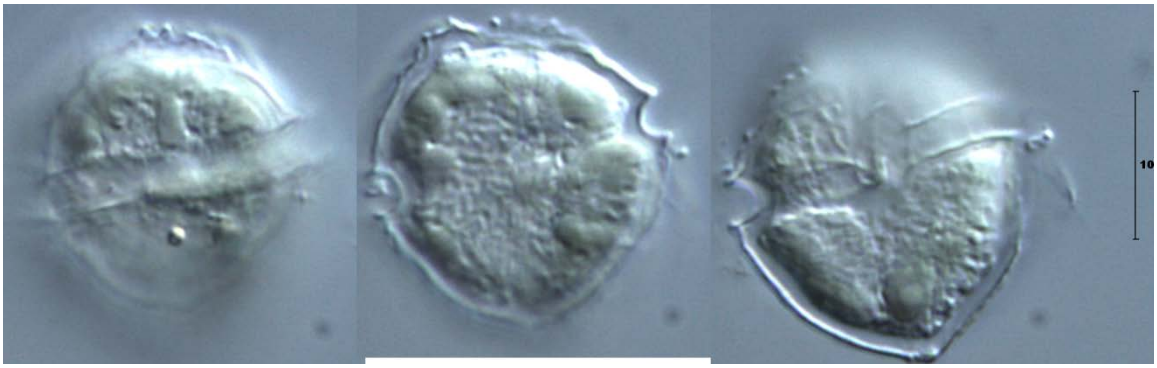


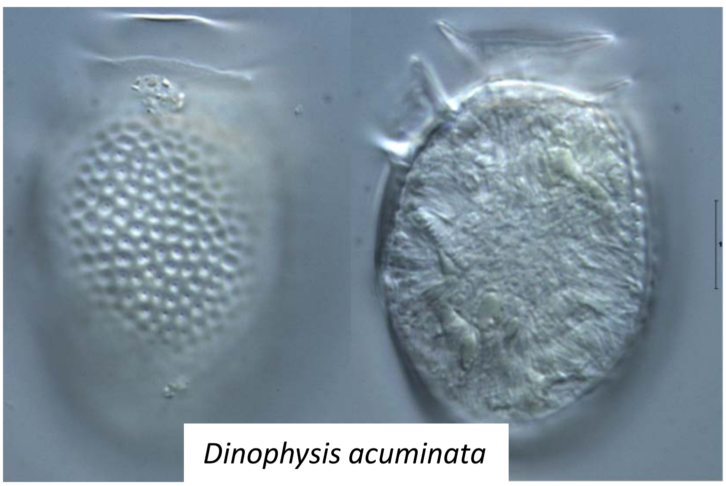
Thalassiosira tenera



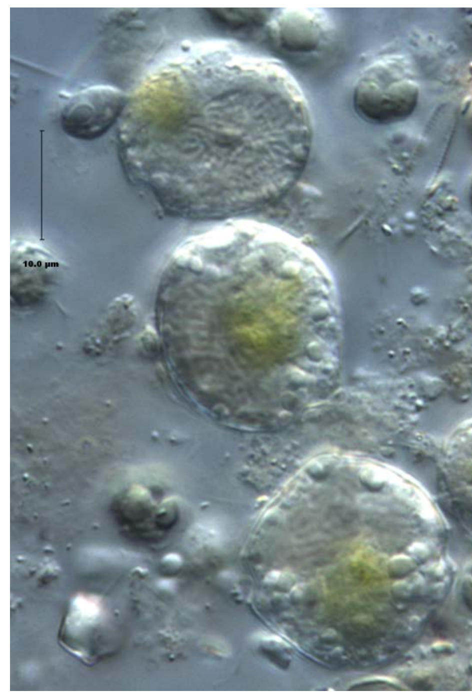
Thalassiosira sp.

Plate 6 Dinoflagellates





Dinophysis acuminata



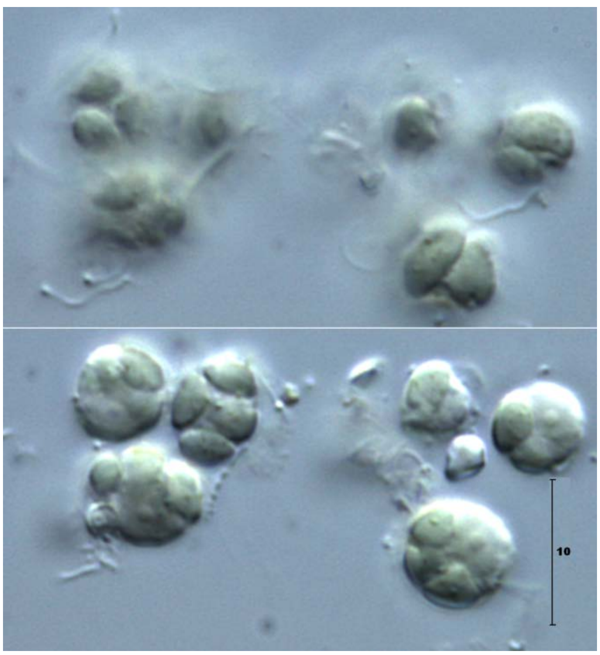
Alexandrium tamarense

Gyrodinium flagellare

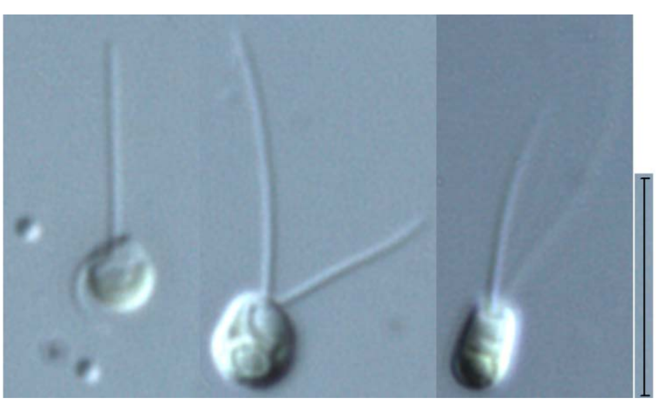
Heterocapsa rotundata

Plate 7 Phytoflagellates

Heterosigma akashiwo

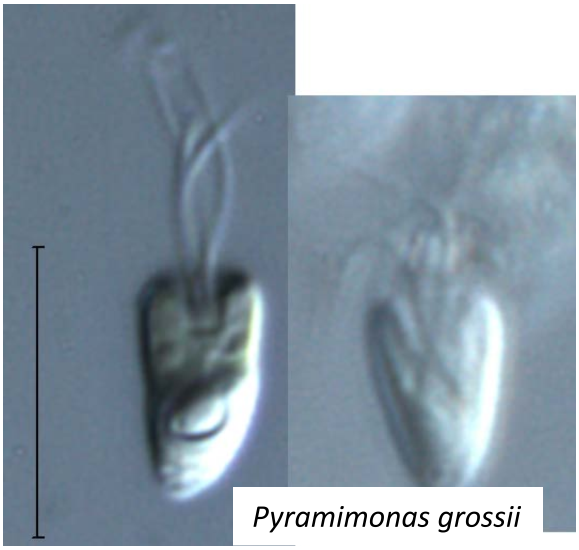


Pseudopedinella pyriformis

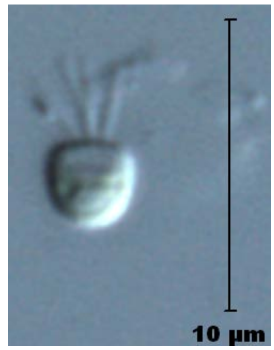


Pseudoscourfieldia marina

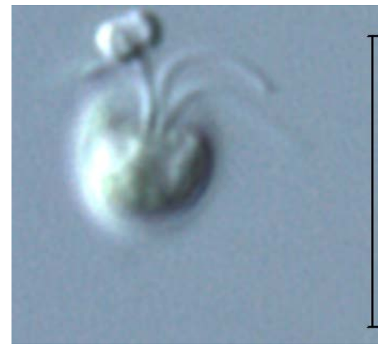
Plate 8   Phytoflagellates



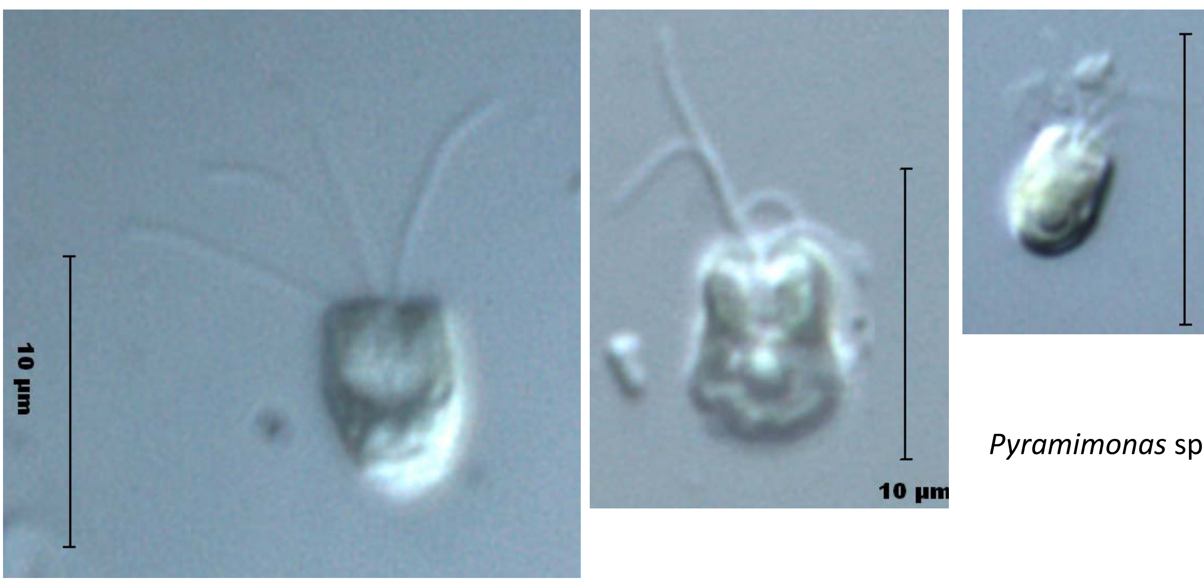
Pyramimonas grossii



Pyramimonas virginica

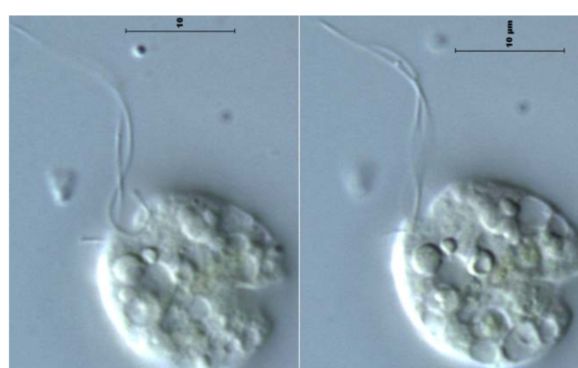


Pyramimonas orientalis



Pyramimonas spp.

Mamiella gilva



Pachysphaera pelagica

Baseline Characterization of Phytoplankton and Harmful Algal Blooms in Barnegat Bay-Little Egg Harbor Estuary, New Jersey (Year Two)

FINAL REPORT

Prepared for

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EXECUTIVE SUMMARY

Barnegat Bay-Little Egg Harbor (BB-LEH) in New Jersey is very susceptible to human-induced eutrophication due to its shallow depth, relatively long flushing time and highly developed surrounding watershed. The Estuary has been classified as a highly eutrophic system (Nixon 1995, Bricker et al. 2007), experiencing episodic recurrences of brown tides and other microalgal blooms, loss of submerged aquatic vegetation, and decline of hard clam stock and harvest.

We carried out a two-year survey of the phytoplankton community in BB-LEH estuary in coordination with New Jersey Department of Environmental Protection (NJDEP)ǿs Bureau of Marine Monitoring during 2011-2013. The study aimed to characterize species composition and spatial and temporal trends in the BB-LEH phytoplankton community, including bloom patterns, dominant species succession, and occurrence of Harmful Algal Bloom (HABs) species. Additional study goals were to compare year-to-year changes on phytoplankton community structure, and to understand the relationships between the changes of species composition and environmental variables. The Year-one report included the species composition of phytoplankton and its seasonal variations from 9 sites from August 2011 to September 2012 (Ren 2013).

This report presents the data on the phytoplankton community from October 2012 to August 2013, based on samples collected at monthly and biweekly intervals from 6 sites throughout BB-LEH. Analysis of phytoplankton included species identification and enumeration, and calculation of cell density and biovolume. In addition, the report includes the results from multivariate analyses on the temporal changes of phytoplankton between Year-one and Year-two, and the relationship between the phytoplankton changes and environmental conditions based on the Year-one and Year-two data.

The number of phytoplankton taxa recorded from the Year-two samples was similar to Year-one. Most common species belonged to five major groups: diatoms (Bacillariophyceae), dinoflatellates (Dinophyceae), cryptophytes (Cryptophyceae), chlorophytes (Chlorophyceae), and chrysophytes (Chrysophyceae). Diatoms made up approximately 50% of the total number of taxa, followed by dinoflagellates. There were differences detected between Year-one and Year-two in regards to species occurrence and dominance among seasons and sites, but species richness and diversity were comparable between the two years. Some species, such as the small and spiny diatom *Chaetoceros* cf. *tenuissimus,* which formed massive blooms in northern Barnegat Bay sites in spring 2012, were not observed at all in 2013. Diatoms were the major components dominating the phytoplankton biomass at all sites during winter-spring in 2012-2013. Picoplankton, pico-coccoids and co-occurring cyanobacteria were numerically dominant in summer at the sites north of Barnegat Bay inlet, coincident with Year-one. In southern Barnegat Bay and Little Egg Harbor, summer phytoplankton in Year-two was dominated by small centric diatoms and lightly silicified diatoms.

Less frequent occurrence and low abundance of harmful species were observed in comparison to Year-one. Major harmful species found in Year-two were *Heterocapsa rotundata* (=*Katodinium rotundatum*) in winter, and *Synechococcus* sp. in summer. For both species, the highest abundance was detected near the mouth of Toms River. *Prorocentrum minimum* was observed at high abundance (10^5~10^6 cells L^{-1}) in Northern Barnegat Bay in Year-one (winter-spring 2011/12), but was not encountered in Year-two. *Prorocentrum* species, including *P. minimum*, *P. triestinum* and *P. micans* were found in a few samples during the study period, but at lower cell density (10^4~10^5 cells L^{-1}) compared to Year-one. In addition, *Pseudo-nitzschia* species were detected in Little Egg Harbor in Year-one with occasional high abundance, but were not encountered in Year-two.

The inter-annual changes of phytoplankton species composition varied in different areas of BB-LEH, likely related to their specific hydrological conditions. In northern Barnegat Bay (BB01), where the water residence time is longest, the phytoplankton community was significantly influenced by Hurricane Sandy. Winter and spring phytoplankton assemblages after the Hurricane were significantly different compared to those from the previous year. The phytoplankton community at BB04 may have been more affected by Toms River with its freshwater discharge and nutrient loading. The summer and winter communities were similar between the two years. And spring and fall community changes were more dynamic, corresponding to temperature changes. In southern Barnegat Bay and Little Egg Harbor, as represented by BB09 and BB12, the phytoplankton community after the Hurricane was more or less distinct from that before the storm. Seasonally, the spring and fall changes were more dynamic compared to other seasons.

Multivariate analysis of all samples from Year-one and Year-two showed significant relationships between phytoplankton species composition and the environmental variables. In addition to salinity and temperature, several nutrient variables were significantly related to the change of phytoplankton community, including total nitrogen (TN), dissolved silica (DSi), total phosphorus (TP), TN:TP ratio, dissolved and total organic carbon (DOC and TOC), as well and dissolved oxygen (DO) and total suspended solids (TSS). High abundance of diatoms was negatively related to DSi in the water column in both years, indicating Si limitation in spring and summer. The dominance of pico-coccoids and cyanobacteria in summer was significantly related to high nutrients, particularly TN and dissolved organic matter, and low concentration of dissolved oxygen in the water column.

The results further confirmed that the change in species composition was sensitive to nutrient input in BB-LEH, and that the phytoplankton community is an important component of water quality monitoring. Our study provides valuable information for the development of indicator species. The two years of phytoplankton data, in combination with water quality data, provide a good starting point for the development of biotic indices for water quality assessment in BB-LEH. Furthermore, data from this study, which include species composition and abundance, biovolume, and carbon biomass, can be useful for related studies on understanding interactions between anthropogenic nutrient loadings, phytoplankton response and food web alteration in BB-LEH, and on modelling development for BB-LEH water quality management. The image and taxonomic documentation generated from this study provides valuable information on phytoplankton taxonomy and data comparison for future studies in BB-LEH and adjacent regions.

Uncertainties and Recommendations

The study showed significant year-to-year differences in phytoplankton assemblages and species succession at different sites in BB-LEH between 2011 and 2013. In addition to nutrient loading, precipitation/drought and hydrology are considered the important factors affecting the inter-annual changes in the phytoplankton community. The study showed that Hurricane Sandy had affected the phytoplankton composition in BB-LEH. How the resulting phytoplankton changes related to associated food web changes is beyond the current scope of work. Further detailed studies are recommended to link system alterations by extreme weather events with the changes of phytoplankton and other biological components, and the effects on system water quality for the long term. Analyses of indicator species are underway for the P-IBI development as part of the scope of work for Year-three. However, a phytoplankton index based on two years of data may inevitably exhibit uncertainty as the estuarine system was altered by the disturbance of the Hurricane. In addition, multivariate analysis including all-season data showed water temperature being a more important factor than most other variables. Future data analyses on data subset by seasons should be conducted to partial out temperature influences and focus more on nutrients and other water quality characteristics. Therefore, more investigations on seasonal variations in phytoplankton communities are recommended to better understand the development of the ecosystem after Hurricane Sandy, and to accumulate a longer term dataset for further qualitative and quantitative analysis of the associations among nutrient loadings, phytoplankton responses and other habitat characteristics, and indicator development.

Brown tide, the bloom of *Aureococcus anophagefferens*, has been a major concern in Barnegat Bay-Little Egg Harbor system. It has occurred episodically since the first confirmed detection in 1995. Like other algal blooms, its formation can be a result of many factors, including hydrological, meteorological, as well as chemical and biological conditions. Therefore, the blooms are usually patchy, and their breakdown can be rather rapid, which makes HAB monitoring a challege. In this study, we used the method of polyclonal antibody labeling and fluorescence microscopic observation and detected low density of *Aureococcus anophagefferens* in southern Barnegat Bay and Little Egg Harbor. An incidence of *Aureococcus anophagefferens* bloom, however, was detected near Sedge Island on June 19, 2013 (4.5 x10^8 cells/L, Bricelj et al. unpublished data). In addition, several other HAB species were recorded in this study including *Prorocentrum minimum*, *Heterocapsa rotundata* (=*Katodinium rotundatum*), *Dinophysis acuminate*, *Pseudo-nitzschia* and *Chaetoceros* species which occurred at high cell density. Even though the detected species and their abundance varied year to year, the study showed their presence in BB-LEH, which is a primary factor indicating the potential for harmful blooms. Continuous monitoring and studies on these HAB species in BB-LEH, including their dominance and blooms and the triggering factors and mechanisms, are recommended.

INTRODUCTION

Background

Phytoplankton responds directly to changes in physical and chemical condition in aquatic systems. The direct effects of nutrient enrichment on phytoplankton in estuaries include excessive growth and biomass, species shifts and frequent noxious and harmful algal blooms (Glibert et al. 2005). These changes in phytoplankton components have significant effects on the organisms at higher trophic levels in the food web. Fish kills and/or reduction of some important fishery resources are often linked, directly or indirectly, to some specific algae, especially harmful algal blooms. The complex interactions between anthropogenic nutrient loadings, phytoplankton species composition, and higher trophic alteration are fundamental to understanding the ecological status of any estuarine and coastal systems.

The Barnegat Bay-Little Egg Harbor Estuary (BB-LEH) is a shallow, poorly flushed system bordered by a highly developed watershed. It is therefore very susceptible to nutrient enrichment. The Estuary has been classified as a highly eutrophic system as determined by application of the National Oceanic and Atmospheric Administration (NOAA) National Estuarine Eutrophication Assessment model (Bricker et al. 2007) and Nixonös Trophic Classification (Nixon 1995, Kennish et al. 2007). The ecological health of the estuary has deteriorated over the last few decades with episodic recurrences of brown tides and other microalgal blooms, loss of submerged aquatic vegetation, and decline of hard clam stock and harvest (Kennish et al. 2010a). A recent USGS report showed that total nitrogen (TN) and total phosphorus (TP) loading from surface runoff for the entire BB-LEH has been increasing, and ranged from 455,000 kg N (in 1995) to 857,000 kg N (in 2010), and from 17,000 kg P (1995) to 32,000 kg P (2010), respectively (Baker et al. 2014). On average, the northern segment accounted for more than half of the annual nutrient loads, 66% for TN and 63% for TP, due to the highly developed watershed. Understanding the relationships between the ongoing nutrient loading (including the forms, concentration and ratios of nutrients) and the changes of phytoplankton community is essential for water quality assessment and management in the BB-LEH Estuary.

We investigated the phytoplankton community in BB-LEH from August 2011 to September 2012 within the scope of work in the Year-one project. Species composition and cell density, seasonal changes of dominant/abundant species, and species succession from nine sites throughout the BB-LEH region were studied and recorded in detail. Several major HAB species and their occurrences and abundance were documented, including some potentially harmful species detected in the north at high abundance. They had not been recorded before this study (Ren 2013). Previous studies as well as monitoring data showed that year-to-year change of phytoplankton community and bloom development varied greatly in BB-LEH, especially concerning harmful algal blooms and brown tide occurrences (Schuster 1999, 2004, Pecchioli et al. 2006). In order to better understand annual and inter-annual changes of phytoplankton in BB-LEH, continuous investigation on the phytoplankton species composition and abundance was carried out at six sites from October 2012 to August 2013, using a monthly-biweekly sampling strategy.

Objectives of Study

The Year-two study aims to 1) continue to characterize phytoplankton species composition and its spatial and temporal trends in the BB-LEH (2012-2013); 2) document bloom patterns and dominant species successions over time (2012-2013; 3) compare year-to-year changes on phytoplankton community structure (2011-2013); and 4) understand the correlations between the changes of species composition and environmental variables (over the two-year study period. In addition, same as in Year-one, we calculated biovolume biomass based on species abundance and biovolume measurements. We further calculated carbon biomass based on biovolume measurements. The overall objective of the study is to provide baseline information on the phytoplankton community in BB-LEH to assist the assessment of current water quality and the development of management tools.

FIELD AND LABORATORY METHODS

Sampling

Cluster analysis based on Year-one phytoplankton community data showed that the 8 sites in BB-LEH can be classified mainly into three groups (Fig. 1). Among all the sites, BB01, BB02, BB05a and BB07a in northern Barnegat Bay can be grouped together, and B09, BB12 and BB14 can be grouped together. BB04a, near the mouth of Toms River, is in general different than the other sites. BB12 and BB14 in Little Egg Harbor were clustered together closely. Accordingly, in the Year-two investigation, six sites, BB01, BB04a, BB07a, BB09, BB10 and BB12, were selected for continuous phytoplankton community analysis. The selected sites span a range of salinity and nutrient regimes along BB-LEH. The locations of the sites are shown in Table 1 and Fig. 1. In particular, BB01 is located at the northernmost end of Barnegat Bay, just south of the Mantoloking Bridge and a USGS monitoring site (USGS01408168). BB04a is located near the mouth of Toms River. BB07a and BB09 are located north and south of Barnegat Bay Inlet. BB10 is located near the adjunction of Barnegat Bay and Little Egg Harbor. And BB12 is located within Little Egg Harbor. All six sites were coordinated with the existing sites of the NJDEP water quality monitoring in BB-LEH (Barnegat Bay LMP QAPP 2013). In year-two, phytoplankton samples were collected at the above-mentioned sites from October 2012 to August 2013. In total, 79 samples were collected from these 6 sites and analyzed quantitatively for phytoplankton species composition.

Phytoplankton sample collections were synchronized with NJDEP water quality grab samplings, and were done via the courtesy of NJDEP Bureau of Marine Water Monitoring. Surface water (<0.5 m) was collected monthly from October 2012 through March 2013, and biweekly from April through August 2013. The samples were preserved with glutaraldehyde to a final concentration of about 0.5%-1% (v/v). Samples were kept dark and cool (~ 4^{o}C) during transportation and prior to analysis. For each sample, three different processes were performed, 1) about 150-250 ml of sample water was dispensed for size-fractionated filtration and whole-community microscopic analysis; 2) one-liter of sample water was settled for further processing for diatom analysis when necessary; 3) about 200-500 ml of water, depending on the biomass, was settled to concentrate to about 20 ml for qualitative and light microscopic observation, if necessary, and for archive purposes. The remnants from 1) were also kept for archive.

Phytoplankton Whole-Community Counts

Phytoplankton samples were size-fractionated by filtering through 0.2 µm, 3 µm and 8 µm pore-size filters. The latter two fractions were stained with 0.03% proflavine hemisulfate. The 0.2 to 3 µm fraction was counted immediately after filtration. The >8 µm fraction was frozen and counted later. Algal identification and enumeration, including soft-algae and diatoms, were done under an epifluorescence microscope (Leica DM L) with blue and green excitation lights and transmitted light. For 0.2 and 3 μm pore-size filters, observations were done under ×1000 magnification. For each filter, at least 5 random fields were counted or until at least 100 cells were counted. If the filter was very sparse, then 50 random fields were counted before stopping. For 8 μm pore-size filters, each filter was observed under three magnifications: First, under ×1000 magnification for phytoplankton <20 μm with the same counting strategy in terms of finishing point; second, under ×400 magnification for larger (>20 μm) phytoplankton with a maximum of 25 random fields when it was sparse; Third, under ×100 magnification to catch some large organisms, which might not have been able to be counted under higher magnifications due to either their large size or sparse density. The method allowed us to be able to examine small size phytoplankton (< 20 μm) under higher magnification (×1000) compared to other methods, e.g. using Palmer-Maloney and/or Sedgewick-Rafter counting cells. The blue and green excitation helps us to differentiate groups of algae when stained with dyes (Dortch et al. 1997, Ren et al. 2009). For samples with high abundance and diversity of diatoms, diatom slides were made separately. Diatoms were analyzed to get the percentage of dominant diatoms, especially the small centric diatoms. Phytoplankton species were identified to the lowest taxonomic level possible. In addition, each common taxon (5% of total cell counts) was documented with images. Biovolumes of common taxa were calculated based on microscope measurements of dimensions and geometric models of phytoplankton (Hillebrand et al. 1999, Olenina et al. 2006). Carbon biomass was calculated based on the biovolume measurements and the cell carbon content for diatoms and non-diatoms from literature (Eppley et al. 1970).

Multivariate Analyses

Cluster analysis

Cluster analysis, using Wardŝ linkage method and Euclidean distance measure, was performed to find groups for classification of sites and seasons with respect to phytoplankton community changes. For Year-one analysis, since three sites (BB04, BB05 and BB07) were shifted after 05/23/2012 (see more information in Ren 2013), in order to exclude the effect of location shifting, only samples collected after the 05/23/2012 were used in the cluster analysis for site classification. Cluster analysis for season classification was conducted on those sites with continuous sample collection from the beginning. Outlier analysis was performed prior to cluster analysis for each dataset.

Nonmetric Multidimensional Scaling

Nonmetric Multidimensional Scaling (NMDS, or NMS) was conducted in the study to establish seasonal and year-to-year changes in phytoplankton community using two years of data from August 2011 to August 2013. The method is an ordination technique, calculating the similarity (dissimilarity) in species composition among each pair of samples. Different from the other classical scaling (e.g. Principal coordinates analysis), NMDS considers only the rank order of the dissimilarity (similarity) instead of their quantitative values. In addition, instead of performing eigenvalue calculations, NMDS uses iterative least-squares stress fitting to obtain the final case configuration from an initial one. Therefore, the number of axes (dimensions) must be specified in advance to final NMDS. Two steps were thus taken for each NMDS analysis, the preliminary runs, and a final run. Preliminary runs were done on each dataset, as the first step, to select the number of dimensions and final stress and to check the final stability (PC-ORD 4.5, McCune and Grace 2002). The information was then used in the second step, the final run of NMDS which was done in Canoco 5. Sample distances were calculated using Bray-Curtis distance measure. One characteristic of NMDS is that it has no precise analytical solution. In Canoco 5, the case configuration was gradually optimized and obtained using an iterative procedure. For this study, the solution of principal coordinates analysis (PCO, also known as metric multidimensional scaling) was used as the starting configuration with no random distortions (perturbations). The variation of case scores along NMDS axes was then maximized by applying a principal component rotation to the scores resulting from the NMDS optimization process (ter Braak and Ġmilauer 2012). A map of the samples was constructed in two or more dimensions, in which relative distance apart of the samples reflects relative similarity in species composition. There has been increasing use of NMDS in recent studies related to biological community changes in aquatic ecosystems (Clarke 1993, Rothenberger et al. 2009, Reynolds et al. 2014).

Principal Components Analysis

Principal Components Analysis (PCA) was performed to seek correlations among environmental variables. This step was used to reduce the number of variables in the water quality dataset but without losing much of the information that was in the original dataset. Solutions based on proportionately more variables will be less stable and the resulting eigenvector coefficients will be less reliable (King and Jackson 1999). More than 20 physical and chemical variables were measured and analyzed in BB-LEH from the NJDEP Water Quality Monitoring Project since summer 2011. More information can be found at http://www.state.nj.us/dep/barnegatbay/bbmapviewer.htm.

Water quality data from the same dates as phytoplankton collections were extracted for this study. Several variables were excluded prior to the data analyses because more than 3/4 (arbitrarily set) of the data values were missing or below limit of detection (LOD). For this reason, variables such as nitrate+nitrite (NO3+NO2), NH3, and ortho-P were excluded from the environmental matrix. For the variables with only few missing data, the following strategies were used to fill in the missing or below LOD data, so that information could be kept: 1) the values below LOD were arbitrarily set to half the detection limit as recommended in several references (Hornung and Reed 1990, Lambert et al. 1991). For missing data, values were artificially set either with the mean values from nearby sites or those from the same month. In addition, TN:TP ratios (in moles) were calculated from TN and TP and included in the environmental matrix. In the end, there were 17 environmental variables for Year-one (Table 2), and 21 for Year-two PCA analyses (Table 3).

Canonical Correspondence Analysis

Canonical Correspondence Analysis (CCA) was conducted to explore the relationship between the phytoplankton species community and environmental factors. For Year-one, phytoplankton community data included samples collected from September 2011 to September 2012. Rare taxa (<5% of total abundance) were excluded in the species matrix prior to the analysis. For Year-two, phytoplankton community data included the samples collected from October 2012 to June 2013. Although phytoplankton species data in Year-two were analyzed through August 2013, water quality data were available through June 2013. All taxa were included in the species matrix, but rare taxa were down weighted during analysis. For both Year-one and Year-two analyses, the species abundance was log transformed prior to the analyses. A few variables were excluded in environmental data matrix because of their strong correlations to other variables, in particular, dissolved oxygen (DO) saturation to DO concentration, alkalinity and conductivity to salinity. In the end, 13 environmental variables were included in Year-one CCA analysis and 11 variables in the Year-two CCA analysis. Datasets for CCA analyses are summarized in Table 4.

Cluster analysis, PCA and first run of NMDS were carried out using PC-ORD 4.5 (McCune and Grace 2002). Final run of NMDS and CCA analyses were performed in Canoco 5.0 (ter Braak and Ġmilauer 2012).

RESULTS AND DISCUSSION

Physical and Chemical Conditions

Water quality monitoring data from October 2012 to June 2013 were downloaded from a NJDEP website: http://www.nj.gov/dep/barnegatbay/bbmapviewer.htm. The variation of water temperature, salinity (ppt), Secchi depth (ft), total nitrogen (TN), total phosphorus (TP) and dissolved silica on the dates of phytoplankton collections from the six sites are illustrated in Fig. 2 and Fig. 3.

There was little difference in water temperature among the six sites (Fig. 2). The lowest temperature was detected in January- February and the highest in late June (August data were not shown). Salinity did not show much seasonal variation and fluctuated from 22 to 30 ppt for most of the sites. Salinity at BB01 and 04a was relatively low and exhibited larger fluctuations compared to the other four sites. The highest salinity at BB01, close to 30 ppt, was detected in November 2012, shortly after the landfall of Hurricane Sandy (Oct. 29, 2012). Salinity at BB04a was the lowest among all sites with the lowest value detected in December 2013, indicating freshwater input from Toms River. Secchi depth, a measure of water clarity, fluctuated between 2 and 6 ft at all sites, not showing much seasonal variation and gradient among different sites.

Recent study showed a strong gradient in watershed nutrient loading, from northern to southern segments (Baker et al. 2014). The northern segment, on average, accounted for over 60% of total annual TN and TP loading, while central and south segments accounted for <20%. However, the changes of TN and TP in BB-LEH did not show much spatial gradient from north to south during the study period. Seasonally, slightly lower concentrations of TN and TP were detected from December 2012 to May- June 2013 compared to other months. Dissolved silica (DSi) exhibited similar seasonal trends to TN and TP. For most sites, the concentration of DSi was low from December 2012 to June 2013, especially in March-April when DSi values were often below the limit of detection (0.01 mg/L). The concentration of DSi increased gradually in summer at all sites. At BB04a, the concentration of DSi showed a spike in December 2012, and because of the replenishment, the concentration of DSi at BB04a was generally higher most of time in comparison to other sites until March-May 2013 when it reached the lowest.

Correlation coefficients among major environmental variables were shown in Table 3. Salinity was highly correlated with conductivity and alkalinity. Turbidity (in TNU) was significantly correlated with total suspended solids (TSS), and Secchi depth measurements. The concentrations of TN, dissolved nitrogen and nitrate+nitrite (NO3+NO2), as well as TOC and DOC were negatively correlated with salinity, indicating freshwater loading, mostly from surface water runoff and groundwater discharge, which was a significant source for nitrogen and organic carbon in the BB-LEH (Wienben and Baker 2009). TP and dissolved P, despite its source from freshwater loading (Baker et al. 2014), had less correlation with salinity, but were more significantly related to temperature and Secchi depth and TSS, possibly suggesting the dominant control of TP and dissolved P concentration in BB-LEH may be biological processes.

Species Composition and Seasonal Changes

A total of 136 taxa were recorded from the Year-two study, and most of the common ones had been recorded from Year-one (Ren 2013). Same as in Year-one, most common species belong to the following five major groups: diatoms (Bacillariophyceae), dinoflatellates (Dinophyceae), cryptophytes (Cryptophyceae), chlorophytes (Chlorophyceae), and chrysophytes (Chrysophyceae). Diatoms comprised the largest number of species, 66, or 50% of the total taxa, followed by dinoflagellates with 24, or 18% of the total. The following diatoms were common at most sites: *Cyclotella* species including *Cyclotella choctawhatcheeana* and *C. atomus*, *Skeletonema 'costatum'*, *Dactyliosolen fragilissimus* and several *Chaetoceros species*. Diatoms, *Asterionellopsis glacialis*, *Leptocylindrus minimus*, *L. danicus*, *Thalassiosira* spp., and *Cylindrotheca closterium* were more often found in middle and southern sites in BB-LEH (BB07, BB09, BB10 and BB12). In addition, some taxa such as *Minutocellus scriptus* (in Year-one, *Minutocellus* sp. 1), *Minidiscus* and *Skeletonema menzelii* emerged in summer at southern sites (BB10, BB12). Main dinoflagellates observed from Year-two included *Gyrodinium flagellare*, *G. estuariale, Heterocapsa triquetra* and *Heterocapsa rotundata* (=*Katodinium rotundatum* in Year-one). They were more often detected in winter-spring and summer at relative low abundance (10^4~$10^5 L^{-1}$) compared to Year-one, except for *H. rotundata*. The highest abundance of *H. rotundata* was found in November at BB04a, > 1.0 x10^6 L^{-1}. High abundance of

Prorocentrum minimum observed from Northern Barnegat Bay in winter-spring 2011/12, did not occur in winter-spring 2012/13. *Prorocentrum,* including *P. minimum*, *P. triestinum* and *P. micans* were found occasionally during the study period, but at very low cell density (10^3 L^{-1}). Cryptophytes have been a consistent component of phytoplankton community during most of the year in BB-LEH. The common taxa included *Hemiselmis* spp., *Plagioselmis* sp., *Teleaulax acuta* and *Leucocryptos marina*. The cell density of major cryptophytes varied with season, and were abundant in spring and summer as observed from BB01, BB09 and BB10 (Fig. 4, Fig. 7 and Fig. 8). Crysophytes were another consistent part of phytoplankton in BB-LEH, mainly comprised of *Calycomonas ovalis*, *C. gracilis*, and *Pseduopedinella pyriformis*. Cyanobacteria became dominant, numerically, in summer (June to August) and in the northern region. Except for the coccoidal cyanobacterium *Aphanocapsa* sp., which had been recorded from Year-one, a skinny oscillatorial *Planktolyngbya* species was observed from most of the sites in April/May. In addition, it is worth mentioning, because of its potential harm, *Synechococcus* sp. was detected at BB04a in May. The typical picoplankton, pico-coccoids being consistent with Year-one, was again detected in summer (June and August) and early fall (October) from sites BB01, 04a, 07a and 09, while at southern sites (BB10 and 12) it was detected in May, but at relatively low density (10^7 L^{-1}).

Cluster analysis with preliminary trial based on all samples from all sites did not show good separation in terms of sites (not shown), possibly because the community dataset contained those from a few months after the Hurricane Sandy when the water system was mixed by the storm. Cluster analysis based on the samples from each site was performed, and showed approximate seasonal separation for most of the sites. Overall, three season groups may be indicated for BB01, BB04a and BB07a: November-December-January/February (winter), March-April-May (spring), and May-June-August (summer). But for southern sites (BB09, BB10 and BB12), the separation could be from November-April, and from April-August. At BB01, 04a and BB07a, the diatoms *Cyclotella choctawhatcheeana, S. costatum* and *Chaetoceros* dominated winter and early spring assemblages. In spring, diatom *D. fragilissimus* started to grow and became dominant in late spring and early summer, while cryptophytes were also abundant. From June to August, the dominant species succession was small size of *Cyclotella atomus*, Pico-coccoids and then cyanobacteria in August (Fig. 4-6). At sites BB09, BB10 and BB12, phytoplankton assemblages were dominated by diatoms during the most of the study period. The succession of the abundant species was as follows: *S. costatum*, *Chaetoceros* spp. *A. glacialis* and *Leptocylindrus* spp in winter-spring →*Thalassiosira* spp., Cryptophytes and Chrysophytes in summer→ small diatoms *Th. Proschkinae*, *Skeletonema menzelli* and *Minutocellus scriptus* in August (Fig. 7-9).

Calculated biovolumes of major phytoplankton taxonomic groups showed that diatoms dominated the phytoplankton biomass from November to May and pico-coccoids dominated the other months at BB01, 04a and 07a (Fig. 10-12). Cyanobacteria, co-occurring with pico-coccoids, although numerically abundant, did not contribute much because of small cell sizes. Diatoms accounted for the majority of phytoplankton biomass at the sites BB09, 10 and 12 during the study period (Fig. 13-15).

Chlorophyll *a* is a routine parameter in water quality monitoring for estimating overall algal biomass. However, knowing the variation and distribution of phytoplankton carbon is important for better understanding carbon dynamics in coastal ecosystems, and for modeling development because carbon is one general currency in biological models (Glibert et al. 2010). Unfortunately, unlike chlorophyll *a*, there is no direct in-situ measurement for phytoplankton carbon biomass, and it is usually estimated from cell biovolume through the microscopic measurements (Mullin et al. 1966, Eppley 1970). The relationship between chlorophyll *a* and carbon biomass varies among different species, sometimes even the same species, but under different physiological conditions due to temperature, light and nutrient stress. It also varies with season in the same region because of different species compositions (Verity et al. 1992). From this study, a significant linear relationship between chlorophyll *a* measurement and calculated carbon biomass was obtained based on Year-two data (Fig. 16).

Year-to-Year Variations in Species Composition

Results from NMDS analysis considering phytoplankton species composition and cell abundance of both Year-one and Year-two samples from four sites were shown in two types of plots for each site of BB01, BB04 (04a), BB09 and BB12: sample scatter plot and sample-species biplot (Fig. 17-20). In both plots, the relative distance between the sample symbols reflects the similarity (points close together) or dissimilarity (points apart) in phytoplankton assemblages (their score values). Arrows between sample dots in sample scatter plots indicate the time series of sample collections. The red arrows mark (approximately) the landfall of Hurricane Sandy

(Oct. 29, 2012). In sample-species biplots, species which contribute most in determining the dissimilarity between groups were superimposed on the ordination diagrams. Each species arrow points in the direction of the steepest increase of species values. The angle between arrows indicates the correlation between individual species.

At BB01, the distance between October and November 2012 indicated large dissimilarity in phytoplankton community before and after the Hurricane at BB01 (Fig. 17a). Phytoplankton assemblages in winter and spring (November to May) following the landfall was significantly distinct from those at the same time in Year-one. The species succession in Year-one proceeded, from winter to spring, from dinoflagellate *Prorocentrum minimum* with diatom *S. costatum* → small Chlorophycean flagellate (*Chlamydomonas* sp. C)→Spine-forming *Chaetoceros*, whereas in Year-two Phytoplankton community was dominated successively by *S. costatum*, Chaetoceros and C. *choctawhatcheeana* from winter to spring. But *A. glacialis*, *D. fragilissimus* and *Leptocylindrus* spp. emerged as influential taxa accounting for the dissimilarity (Fig. 17b). Most of those diatoms, except for *C. choctawhatcheeana* had been detected in Year-one from southern sites with higher salinity. It is evident that the phytoplankton community at BB01 was affected by Hurricane Sandy which pushed more salt water to that part of Barnegat Bay, as shown by salinity in the November-December of 2013 (Fig. 2). Salinity dropped back to pre-hurricane level (~20 ppt) in January. However, most diatoms remained abundant through March-April, particularly *D. fragilissimus,* which became dominant but looked pale and stressed in April. Phytoplankton community in summer (June to August 2013) aggregated more into summer 2012, showing more similarity in phytoplankton between these two years, comprised of pico-coccoids, small *Cyclotella* species and cyanobacteria (Fig. 17a).

At BB04, summer and winter communities were similar between these two years, as shown by congregated samples in Fig. 18. But the changes were more dynamic during spring (such as, from March to June in 2013 as indicated by 1303 to 1306 in Fig. 18) and fall (October-November-December in 2012 as indicated by 1210-1212 in Fig. 18), corresponding to the change of temperature. High dissimilarity in phytoplankton between the month before and after the Hurricane was indicated, but it was not exceptionally high compared to the changes among other months (e.g. November to December, or March to April). The massive blooms of the small green alga *Chlamydomons* sp. *C* and the small diatom *Chaetoceros*, *Ch*. cf. *tenuissimus* recorded in spring and summer of Year-one, were not observed in Year-two, which largely contributed to the dissimilarity, especially in June for *Ch*. cf. *tenuissimus* (Fig. 18, lower panel). Different to BB01, phytoplankton community changes at BB04 were more likely affected by freshwater discharge, as well as nutrient loading, from Toms River. The Hurricane must have pushed the high salinity water to the area as it did to BB01. However, when the collection took place three weeks later, salinity fell back to the same range as before the storm. Variations in freshwater discharge as well as the concentration, forms and ratios of nutrients and organic matter in discharge will surely affect seasonal and inter-annual changes of phytoplankton growth and species composition at this site.

At BB09 the phytoplankton community after the Hurricane was distinct from that before the Hurricane (Fig. 19, Fig. 20). The site is located right below the Barnegat Bay Inlet. The change of phytoplankton was more dynamic, likely resulting from fast water exchanges with coastal water via Barnegat Bay Inlet (Defne and Ganju 2014). BB12 is located in Little Egg Harbor. The difference in the phytoplankton community between the two years was not as large as in BB09, but still evident (Fig. 20). Within each year, the change of the community was larger during spring and fall in comparison to winter and summer, correlating with temperature.

Variations in Species Composition in Relation to Environmental Variables

Sample-environmental variable biplots and species-environmental variable biplots (both axes 1 and 2), with score scaling focused on species scores, were generated from CCA, considering all data from Year-one and Year-two (Fig. 21 and Fig. 22). In the sample-environmental variable biplot, the distance between the sample symbols indicates the dissimilarity (similarity) of their species composition as measured by their chi-square distance. Environmental variables are represented by arrows in the plots. Each arrow points in the direction of the steepest increase of that environmental variable value (ter Braak and Smilauler 2012).

Significant relationships between phytoplankton species and the environmental variables were obtained in Year-one and Year-two, as indicated by Permutation tests with the results on all axes of $P=0.002$. The CCA explained a cumulative 79% and 68%, respectively, of the variation in the species-environmental relationships in Year-one and Year-two as shown by explained fitted variations in Table 5. The variables explaining most variation in phytoplankton community changes in Year-one were salinity, TN, water temperature, dissolved silica, DOC, DO, and TP (Table 5). In Year-two, the most explanatory variables included water temperature, DO, dissolved Si, TOC, TN and TP (Table 6). Salinity was the strongest variable in Year-one correlated with the change in species composition; however, it became a much less controlling factor in Year-two. Samples in Year-two were more congregated compared to Year-one, suggesting overall less temporal and spatial variation in species composition and, probably indicating the system was mixed due to Hurricane Sandy.

The species ï environmental variables diagrams summarize the variation of species composition in relation to the environmental variables. The 30 species with highest weight were shown. Salinity was the factor explaining most of the variation in phytoplankton abundance and species composition in Year-one. Diatom species showed strong correlation with the salinity gradient, with C. *choctawhatcheeana* and small *Chaetoceros cf. tenuissimus* in the spring in the northern area, as indicated by intermediate temperature and low salinity in the diagram. As salinity increased (toward southern sites), diatom assemblages were abundant with *Cerataulina pelagica*, *A. glacialis*, *Thalassionema*, *Leptocylindurs minimus*, *Skeletonema costatum* and small *Cyclotella stomus*. In summer and early fall, phytoplankton was dominated by pico-coccoids and cyanobacteria in northern part with relatively low salinity, while the diatoms *Thalassiosira proschkinae* and *Cylindrotheca closterium* were abundant in southern part of Barnegat Bay and Little Egg Harbor.

In Year-two, diatom distributions along the salinity gradient were still exhibited but relationships were not as strong as in Year-one. Due to the water mixing caused by Hurricane Sandy, the distribution of phytoplankton species did not show as much gradient from north to south in winter and spring in Year two. Species like *D. fragilissimus*, *A. glacialis*, *Thalassiosira* spp. had been detected abundant in northern area. Temperature emerged as the factor most closely associated with community changes, which was expected as the dataset included seasonal changes.

In both years, high abundance of diatoms was associated with lower dissolved Si (DSi) in the water column in winter and spring. This is not uncommon in estuarine ecosystems as shown by other studies (Conley and Malone 1992, Rothenberger et al. 2009). Dissolution of biogenic silica, mainly from diatom frustules buried in the sediment, can be a significant source for silicon restoration in the water column in shallow estuarine systems like Barnegat Bay (Loucaides et al. 2008). During the winter-spring period, regenerated Si was rapidly taken up by diatom populations; therefore DSi remained low. When temperature increased from spring to summer, the regenerated Si may not have been sufficient to match the increasing demand for Si from growing diatom populations. As a result, diatom growth was suppressed due to Si limitation. In the meantime, pico-coccoids and small coccoidal cyanobacteria, with no need for Si, grew rapidly. In addition, they could absorb and take up nutrients more efficiently under low N and P concentrations because of their size and shape (Gobler et al. 2011), which enables them to outcompete large-cell or more heavily silicified diatoms and became dominant in summer and early fall. Diatoms associated with summer picoplankton were found mostly to be small centric diatoms such as *Cyclotella atomus*, *Th. proschkinae,* and very lightly silicified ones, such as *Minutocellus scriptus* and *Phaeodactylum ? triconutum*. Dissolved Si in the water column was gradually accumulated in summer while diatom uptake decreased, as indicated by the positive relationship between dissolved Si and pico-coccoids/cyanobacteria abundance. One major consequence of excessive nutrient input, mainly N and P, in estuarine and coastal areas is the increase of Si limitation (Turner et al. 1998, 2003). It is one of the driving factors to promote a shift in dominance from diatoms to flagellates and cyanobacteria, as well as other non-diatom algae, some of which can be harmful to other organisms and water quality.

The phytoplankton community was significantly related to concentrations of TN and TP (Fig. 21, 22). The dominance of pico-coccoids and cyanobacteria in summer and its correlation with high TN and dissolved organic matter and low dissolved oxygen is coincident with other studies (Rothenberger et al. 2009, Gobler et al 2011). These studies showed that brown tide alga, *Aureococccus anophagefferens*, with similar size and shape as pico-coccoids in this study, outcompete co-existing phytoplankton at elevated levels of dissolved organic matter and turbidity and low dissolved inorganic nitrogen (Gobler et al. 2011). During the Year-one study, we detected low density (10^5~10^6 cells L^{-1}) of *A. anophagefferens* from sites 9 and 12. However, it did not shown up in the counts due to its low abundance. The dominance of dinoflagellates in winter-early spring, such as *P. minimum* in year-one, and *H. rotundata,* was coincident with some previous studies of Barnegat Bay and other mesohaline regions (Springer et al. 2005, Mountford 2013). The occurrence of *P. minimum* in Year-one was positively related to TN:TP ratios.

SUMMARY AND CONCLUSIONS

We investigated the temporal and spatial distribution of the phytoplankton community in BB-LEH from October 2012 to August 2013. We compared changes in phytoplankton assemblages between year-one (August 2011-September 2012) and year-two (October 2012-August 2013), and explored the effects of a variety of environmental variables on species composition. Significant relationships between phytoplankton community and environmental variables were indicated from both year-one and year-two analyses. Water quality parameters including TN, TP, dissolved Si, TN:TP, TOC and DOC, TSS and DO are strongly associated with the variation of phytoplankton species composition. The results further confirmed that the change in species composition was sensitive to nutrient input in BB-LEH, and that phytoplankton community is an important component of water quality monitoring. Our study provides valuable information for the development of indicator species. Furthermore, the two years of data, in combination of water quality data, provide a good starting point for the development of biotic indices for water quality assessment in BB-LEH. The inter-annual changes of phytoplankton species composition was significantly influenced by Hurricane Sandy. Our study provided baseline information on phytoplankton composition in the post-hurricane BB-LEH ecosystem. In addition, data from this study, which include species composition and abundance, biovolume and carbon biomass can be useful for other related studies, especially for the development of BB-LEH ecosystem models.

Major Findings of This Study

Diatoms became the major components of the phytoplankton community and biomass, and were numerically abundant all sites during most of the study period. Most diatom species recorded from year-two had been found in year-one samples. There were detected differences between Year-one and Year-two in regards to the species occurrence and dominance among seasons and sites, but the species richness and diversity were comparable between these two yeas. Some species, such as small and spiny diatom *Chaetoceros* cf. *tenuissimus* forming massive blooms in northern Barnegat Bay sites in spring 2012, were not observed at all in 2013. Despite the similar number of dinoflagellate taxa we recorded, their frequency and abundance were relative low compared to year-one. High abundance of *Prorocentrum minimum* observed from Northern

Barnegat Bay in previous winter did not occur in Year-two. But *H. rotundata* found in November at BB04a reached the cell density of > 1.0 x10^6 L^{-1}.

Cluster analysis of the samples from each site showed seasonal changes in species composition for most of the sites. For BB01, BB04a and BB07a, the development of phytoplankton community could be separated into winter (November-December-January/ February), spring (March-April-May), and summer (May-June-August). The dominant species proceeded from a mixture of *C. choctawhatcheeana, S. costatum* and *Chaetoceros* → *D. fragilissimus* and cryptophytes→ Pico-coccoids, Cyanobacteria and small *Cyclotella* species. For southern sites (BB09, BB10 and BB12), the seasonal separation was shown between November-early April and late April-August. The abundant species in the first half of the study period were a mixture of several diatoms, including *S. costatum*, *Chaetoceros* spp. *A. glacialis*, *Leptocylindrus* spp and *Thalassiosira*. In the second half of the period, phytoplankton in abundance was the small and lightly silicified diatoms *Th. proschkinae*, *Skeletonema menzelli* and *Minutocellus scriptus*.

Ordination analysis showed that phytoplankton community composition was significantly influenced by Hurricane Sandy. The largest change in the phytoplankton community was found at BB01 where the water residence time is the longest. Consequently, the 2013 winter and spring phytoplankton assemblages after the Hurricane were significantly different than those from the previous year. The phytoplankton community at BB04 may have been more affected by Toms River with its freshwater discharge and nutrient loading. In southern Barnegat Bay and Little Egg Harbor, as represented by BB09 and BB12, the change of the phytoplankton community was more dynamic, influenced by water exchange via Barnegat Bay inlet.

Multivariate analysis of all samples from Year-one and Year-two showed significant relationships between phytoplankton species composition and the environmental variables. Salinity appeared to be the most important variable in Year-one controlling the distribution of the phytoplankton composition; however, it became a much less important factor in Year-two. This difference may have been influenced by Hurricane Sandy, when more salt water was pushed to the north of BB, and retained for a considerably long time due to low turnover rate in the area. In addition, temperature was one of the strongest variables, which was expected in the all-season dataset. Several nutrient variables were significantly related to the change of phytoplankton community, including TN, dissolved silica, TN:TP, DOC, DO, and TP in year-one, and DO, dissolved Si, TOC, TN and TP in year-two. The distribution of diatoms showed a strong relationship with the salinity gradient in year-one. High abundance of diatoms was associated with lower dissolved Si in the water column in both years, indicating Si limitation in spring and summer in the system. The dominance of pico-coccoids and cyanobacteria in summer was significantly related to high nutrients, particularly TN and dissolved organic matter, and low concentration of dissolved oxygen in the water column.

Recommendations for Future Studies

The algal dataset, together with water quality monitoring data, provides us a good and ideal starting point to identify indicator species and develop a phytoplankton index of biotic integrity (P-IBI) for BB-LEH. Analyses of indicator species are underway for the P-IBI development, as part of the scope of work for Year-three of the NJDEP sponsored Barnegat Bay Research Program. Meanwhile, data analyses showed significant year-to-year differences in phytoplankton assemblages and species succession, likely due to effects of Hurricane Sandy. As a result, a phytoplankton index based on two years of data alone may inevitably exhibit uncertainty because the estuarine system was altered by the disturbance of the Hurricane. In addition, future data analyses on data subset by seasons should be conducted to partial out temperature influences and focus more on nutrients and other water quality characteristics. Further investigation and monitoring of phytoplankton and harmful algal blooms are therefore recommended to better understand and quantify the relationships between phytoplankton community change and the environmental factors, especially nutrients, in the post-Sandy BB-LEH system. For future analysis, it is necessary to include other factors in the analysis, such as watershed development, land use, freshwater discharge, precipitation, and turnover rate, in order to better understand temporal, spatial and inter-annual changes of the phytoplankton community in BB-LEH.

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LIST OF TABLES

Tables

Table 1. Sites of phytoplankton sample collection in Barnegat Bay-Little Egg Harbor from October 2012 to August 2013 (highlighted).

Site ID	Longitude	Latitude	Site description
BB01	-74.052222	40.04	Barnegat Bay at Mantoloking
BB02	-74.09847	39.97762	Barnegat Bay between Silver Bay and Goose Creek
BB04a	-74.14069	39.93289	Barnegat Bay near the Mouth of Toms River
BB05a	-74.1094237	39.9157764	Barnegat Bay above Cedar Creek
BB07a	-74.1571172	39.8012861	Barnegat Bay below Oyster Creek and above Barnegat Inlet
BB09	-74.14792	39.74262	Barnegat Bay below Barnegat Inlet and close to Long Beach
BB10	-74.20653	39.66095	Barnegat Bay by Route 72 Bridge
BB12	-74.26875	39.58151	Barnegat Bay in Little Egg Harbor
BB14	-74.29737	39.51123	Little Egg Harbor Inlet near Beach Haven Heights

Table 2: Correlation among environmental variables from PCA based on year-one data (values in bold indicate significance at P < 0.01)

	WaterT	DO (mg/l)	DO Sat %	pH	Salinity (ppt)	Turbdty (NTU)	SC (uS/cm)	TSS (mg/l)	Chl *a* (ug/l)	TN (mg/l)	Dis N (mg/l)	TP (mg/l)	DOC (mg/l)	Alk (mg/l)	Tot Si (mg/l)	Dis Si (mg/l)	TN: TP (in mole)
WaterT	1																
DO (mg/l)	**-0.89**	1															
DO Sat %	**-0.62**	**0.88**	1														
pH	0.05	0.02	0.12	1													
Salinity	-0.24	-0.02	-0.1	0.4	1												
Turbidity	0.17	-0.16	-0.17	0.25	0.13	1											
SC	-0.27	0.08	-0.08	0.4	**0.99**	0.13	1										
TSS	-0.2	0.07	-0.04	0.11	0.41	0.41	0.42	1									
Chl *a*	0.35	-0.16	-0.04	-0.02	-0.4	0.26	-0.4	-0.15	1								
TN	**0. 9**	-0.35	-0.18	-0.26	**-0.7**	0.1	**-0.76**	-0.36	0.53	1							
Dis N	0.27	-0.21	-0.18	-0.1	-0.16	0.01	-0.21	-0.09	0.01	0.25	1						
TP	**0.69**	-0.63	-0.47	0.14	0.09	**0. 6**	0.08	0.13	0.36	0.41	0.06	1					
DOC	0.42	-0.28	-0.19	-0.11	**-0.49**	0.1	-0.5	-0.17	0.34	**0. 8**	0.34	0.23	1				
Alk	-0.25	-0.07	-0.07	0.45	**0.98**	0.17	**0.97**	0.44	-0.36	**-0.73**	-0.19	0.1	-0.48	1			
Tot Si	0.35	-0.21	-0.12	-0.05	-0.31	0.41	-0.32	-0.05	0.45	0.52	0.25	0.4	0.46	-0.29	1		
Dis Si	0.4	-0.23	-0.12	-0.27	**-0.**	0.06	-0.51	-0.24	0.5	**0.61**	0.23	0.32	0.49	-0.48	**0.76**	1	
TN:TP	-0.38	0.43	0.27	-0.22	-0.47	-0.35	-0.45	-0.3	-0.13	0.13	0.07	-0.57	0.05	-0.49	-0.05	-0.01	1

Table 3: Correlation among environmental variables from PCA based on year-two data (values in bold indicate significance at P < 0.01)

	WaterT	DO_mg/l	DO_Sat%	pH	Salinity	Turbdty	SC_uS/cm	Secchi_f	TSS_mg/l	Chla	TN_mg/l	Dis_N	NO2+NO3	Dis_NH3	TP_mg/l	Dis_P	DOC	TOC	Alk	Dis_ Si	TN:TP
WaterT	1.00																				
DO_mg/l	**-0.88**	1.00																			
DO_Sat%	-0.26	**0.60**	1.00																		
pH	-0.08	0.06	0.36	1.00																	
Salinity	-0.02	-0.13	0.16	**0.74**	1.00																
Turbdty	-0.08	0.11	0.15	0.10	0.18	1.00															
SC_uS/cm	-0.06	-0.09	0.18	**0.76**	**1.00**	0.19	1.00														
Secchi_f	-0.13	-0.05	-0.24	-0.10	-0.09	**-0.43**	-0.09	1.00													
TSS_mg/l	0.14	-0.11	0.07	0.27	0.38	**0.70**	0.37	**-0.52**	1.00												
Chla	0.10	0.01	-0.01	-0.01	-0.34	0.07	-0.33	-0.30	0.00	1.00											
TN_mg/l	0.26	-0.17	-0.24	**-0.59**	**-0.59**	0.22	**-0.60**	-0.25	-0.01	0.32	1.00										
Dis_N	0.19	-0.14	-0.24	**-0.74**	-0.54	0.26	**-0.56**	-0.09	-0.04	-0.05	**0.73**	1.00									
NO2+NO3	-0.24	0.31	-0.04	**-0.78**	**-0.67**	-0.04	**-0.68**	0.15	-0.26	-0.05	**0.51**	**0.68**	1.00								
Dis_NH3	0.05	-0.11	-0.05	0.01	0.21	0.30	0.20	-0.18	0.28	-0.27	0.14	0.29	0.04	1.00							
TP_mg/l	**0.45**	**-0.42**	-0.14	0.11	0.25	**0.49**	0.23	-0.61	**0.54**	0.09	**0.41**	0.20	-0.29	0.29	1.00						
Dis_P	**0.43**	**-0.52**	-0.30	-0.05	0.37	0.17	0.35	-0.24	0.27	-0.32	0.15	0.32	-0.16	**0.52**	**0.66**	1.00					
DOC	**0.49**	-0.28	-0.15	**-0.48**	-0.71	-0.10	**-0.72**	-0.17	-0.20	0.36	**0.61**	**0.50**	0.20	-0.10	0.19	-0.06	1.00				
TOC	**0.49**	-0.24	-0.03	-0.36	**-0.64**	-0.07	**-0.65**	-0.17	-0.21	0.34	**0.58**	0.35	0.18	-0.22	0.20	-0.15	**0.85**	1.00			
Alk	-0.06	-0.12	0.12	**0.75**	**0.96**	0.22	**0.97**	-0.12	**0.41**	-0.34	**-0.57**	**-0.53**	**-0.71**	0.19	0.31	**0.40**	**-0.68**	**-0.65**	1.00		
Dis_Si	**0.46**	-0.40	-0.37	**-0.51**	**-0.44**	-0.03	**-0.47**	-0.24	0.05	0.22	**0.54**	**0.52**	0.31	-0.02	0.36	0.27	**0.56**	**0.41**	**-0.42**	1.00	
TN:TP	-0.16	0.23	-0.08	**-0.75**	**-0.76**	-0.23	**-0.77**	0.31	-0.39	-0.03	0.36	**0.52**	**0.87**	-0.10	**-0.48**	-0.33	0.28	0.24	**-0.80**	0.36	1.00

Table 4: Summary of data sets for CCA analyses.

	Year-one	Year-two
Collection duration	August 2011-September 2012	October 2012-June 2013
Collection sites	8	6
# of samples	134	67
# of species	55	89
Environmental variables	14	11

Table 5: Explanatory power and the strength of the relationships between phytoplankton species composition and environmental variables, evaluated separately by the significance of the first CCA axis, based on Year-one data.

Environmental variable	Explains %	F_ratio	P
Salinity	7.8	11.2	0.002
TN_mg/l	6.2	8.7	0.002
Water temperature (^{o}C)	5.2	7.2	0.002
TN:TP (in mole)	4.4	6.1	0.002
Dissolved_Si	4	5.6	0.002
DOC_mg/l	3.7	5.1	0.002
DO_mg/l	3.6	4.9	0.002
TP_mg/l	3.5	4.7	0.002
TSS mg/l	3.3	4.5	0.002
Total Si	3.1	4.3	0.002
Chla_ug/l	2.5	3.4	0.002
Turbidity (NTU)	2.4	3.3	0.002
pH	2.2	2.9	0.002
Dis_N_mg/l	0.8	1	0.376

Table 6: Explanatory power and the strength of the relationships between phytoplankton species composition and environmental variables, evaluated separately by the significance of the first CCA axis, based on Year-two data.

Environmental variable	Explains %	F_ratio	P
Water temperature (^{o}C)	8.6	6.1	0.002
DO_mg/l	7.3	5.1	0.002
Dissolved Si_mg/l	4.8	3.3	0.002
TOC mg/l	4.6	3.2	0.002
TN_mg/l	4.4	3	0.002
TP_mg/l	4.1	2.8	0.002
TSS mg/l	3.2	2.2	0.004
Salinity (ppt)	3.1	2.1	0.002
TN:TP (in mole)	2.7	1.8	0.01
Chlorophyll *a* (ug/l)	2.6	1.7	0.016
Dis NH3_mg/l	2.2	1.5	0.046

LIST OF FIGURES

Figures

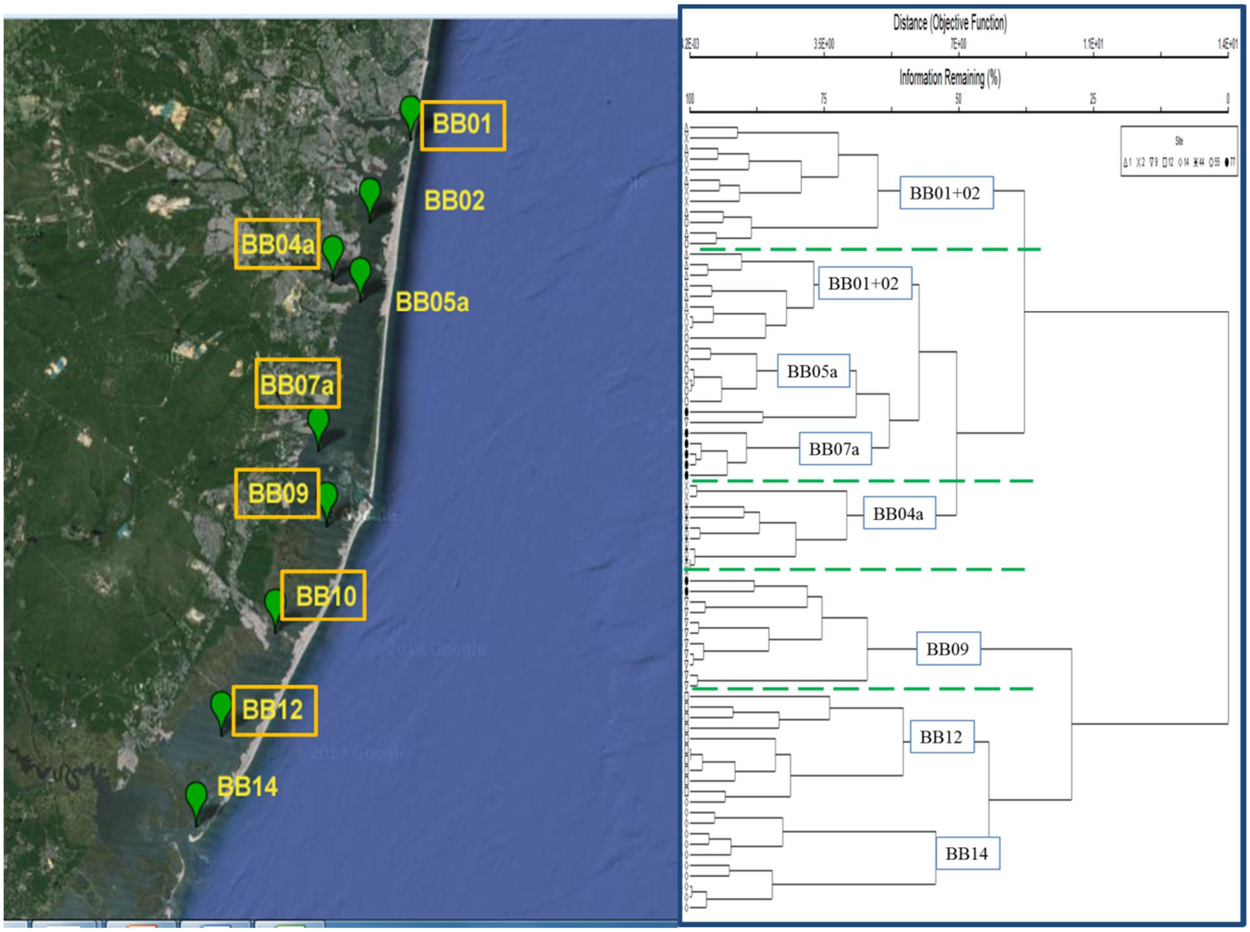


Fig. 1. **Left panel**: Map of sites for phytoplankton sample collection 2011-13. Samples from six sites (framed) were collected from 2012-2013. Note BB04a, BB05a and BB07a were shifted from BB04, BB05, BB07 (not shown) after May 2012. **Right panel**: Cluster analysis of sites based on 2011-12 data (8 sites with exclusion of BB10).

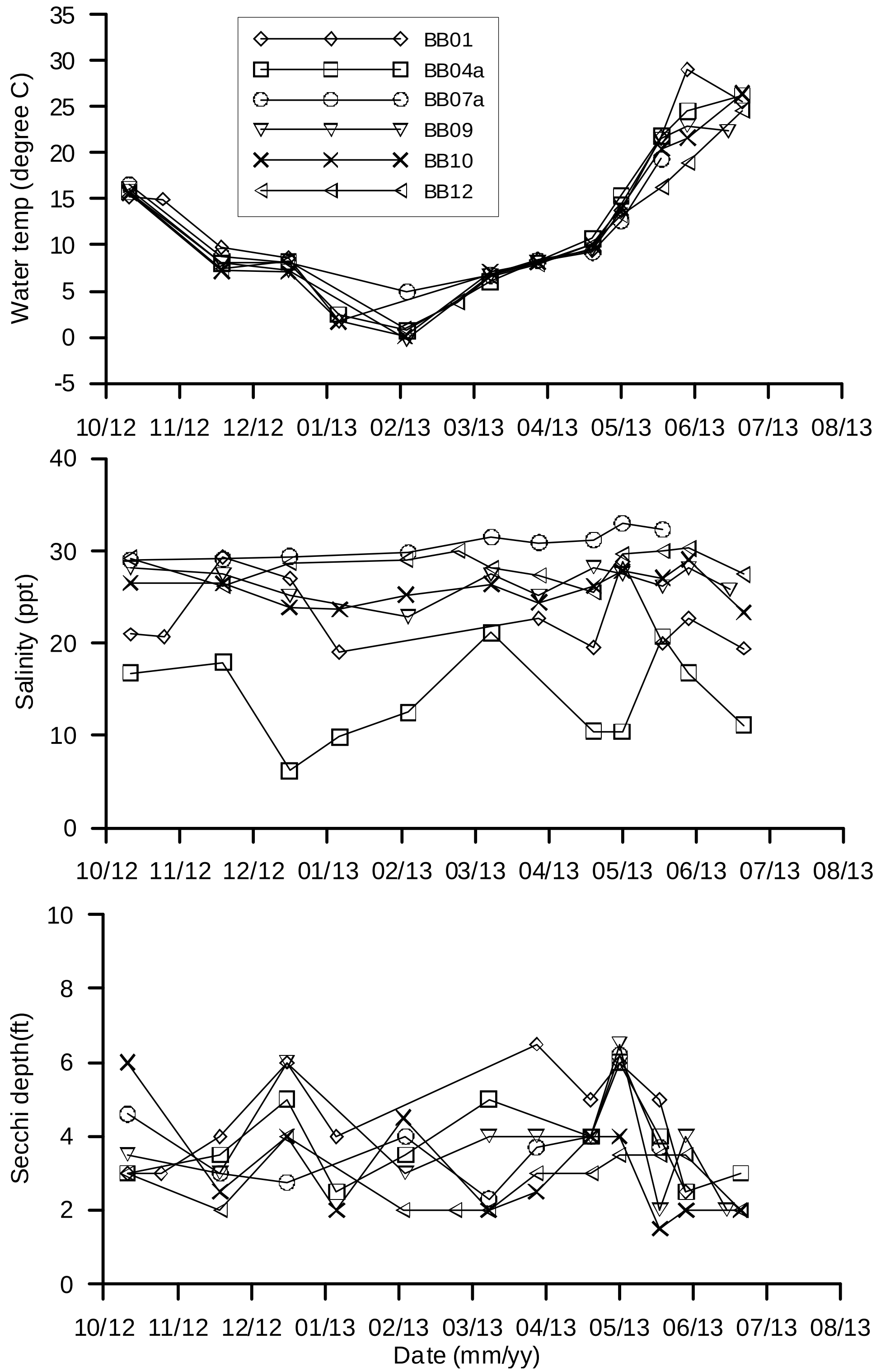


Fig. 2. Change of water temperature, salinity and Secchi depth at phytoplankton collection sites in BB-LEH from October 2012 to August 2013. Data from NJDEP water quality monitoring, http://www.nj.gov/dep/barnegatbay/bbmapviewer.htm.

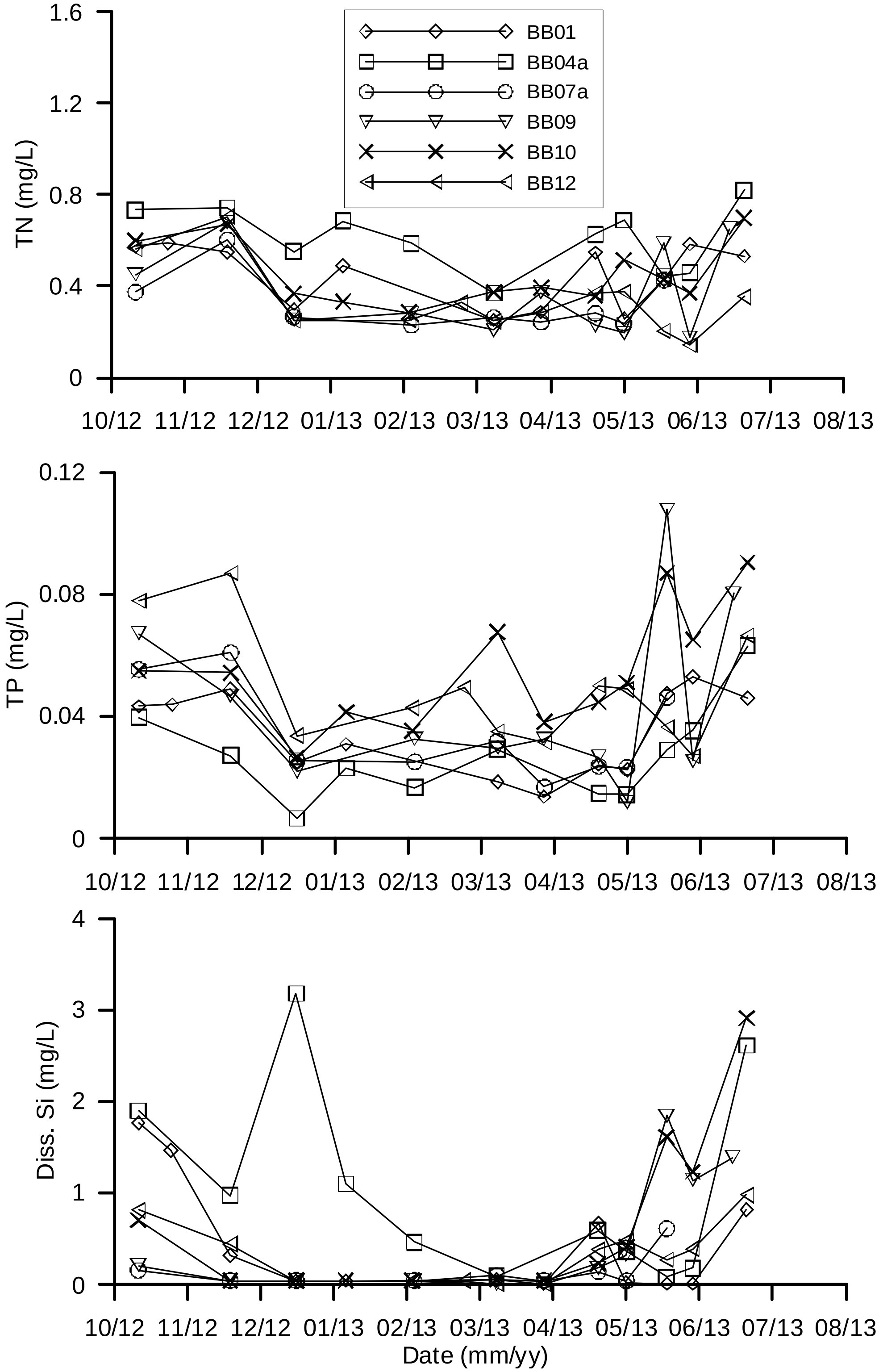


Fig. 3. Change of total nitrogen (TN), total phosphorus (TP) and dissolved Si (DSi) at phytoplankton collection sites in BB-LEH from August 2011 to September 2012. Data from NJDEP water quality monitoring: http://www.nj.gov/dep/barnegatbay/bbmapviewer.htm.

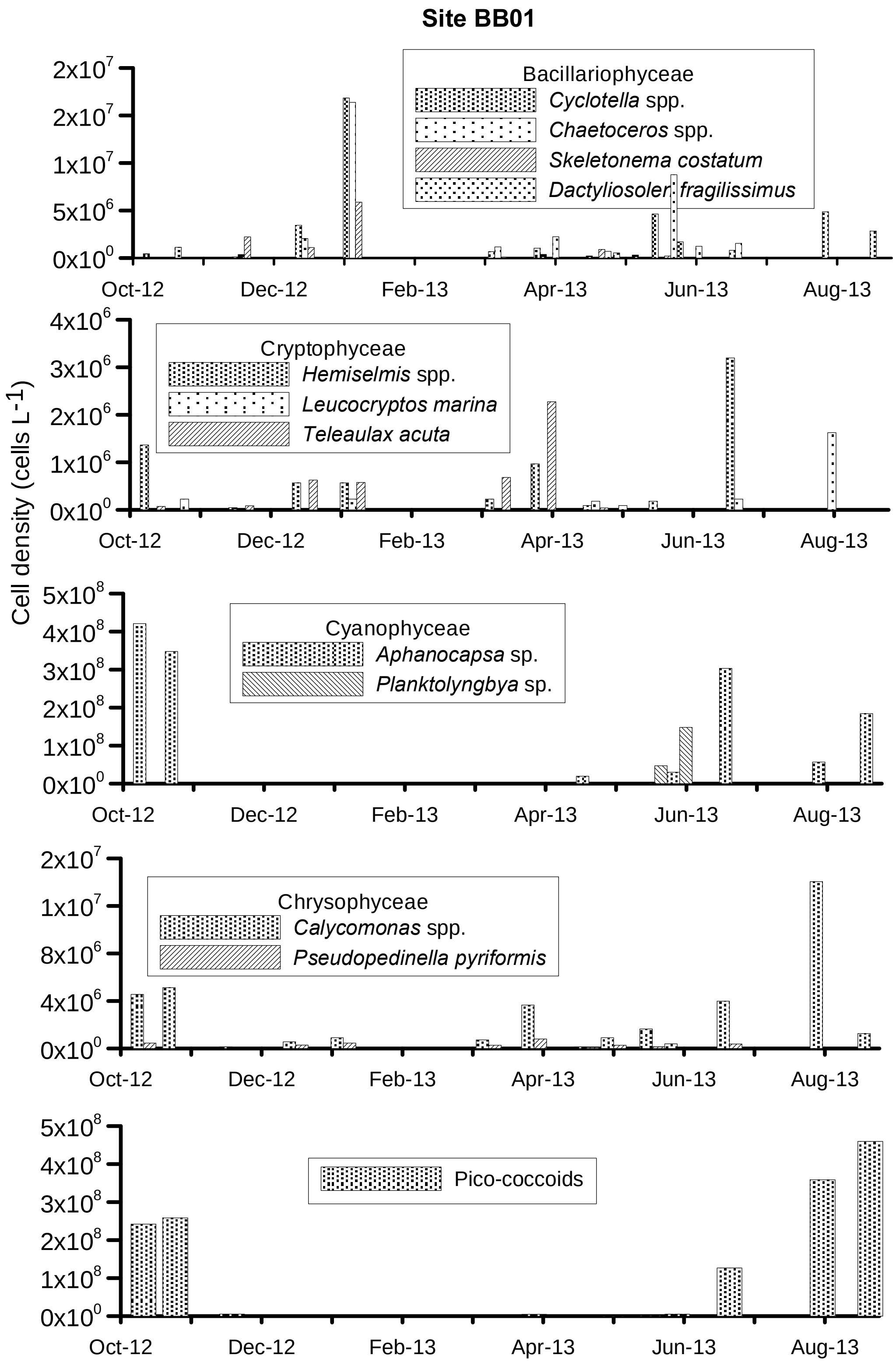


Fig. 4. Abundance and seasonal changes of some dominant species at site BB01 from October 2012 to August 2013.

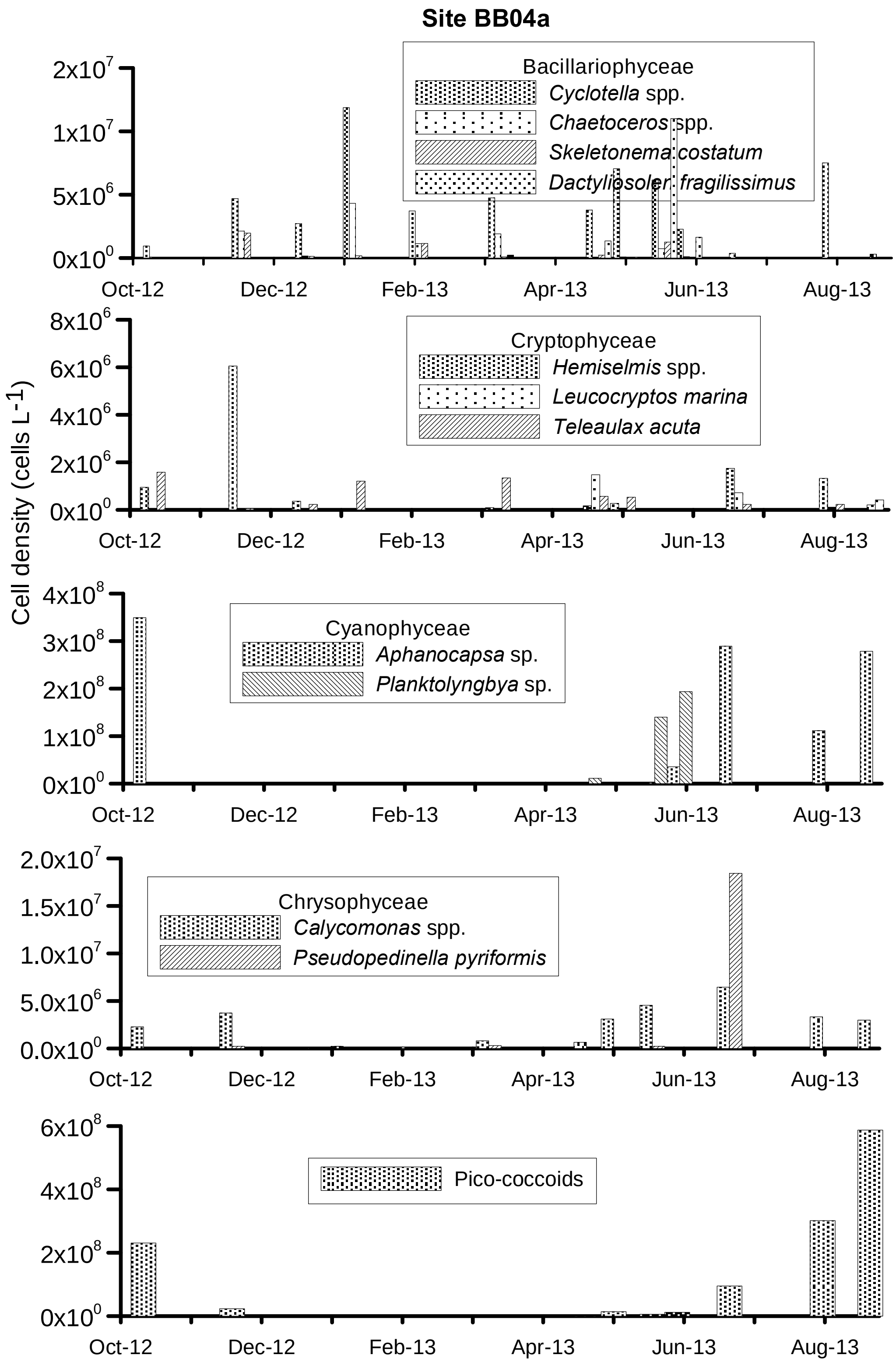


Fig. 5. Abundance and seasonal changes of some dominant species at site BB04a from October 2012 to August 2013.

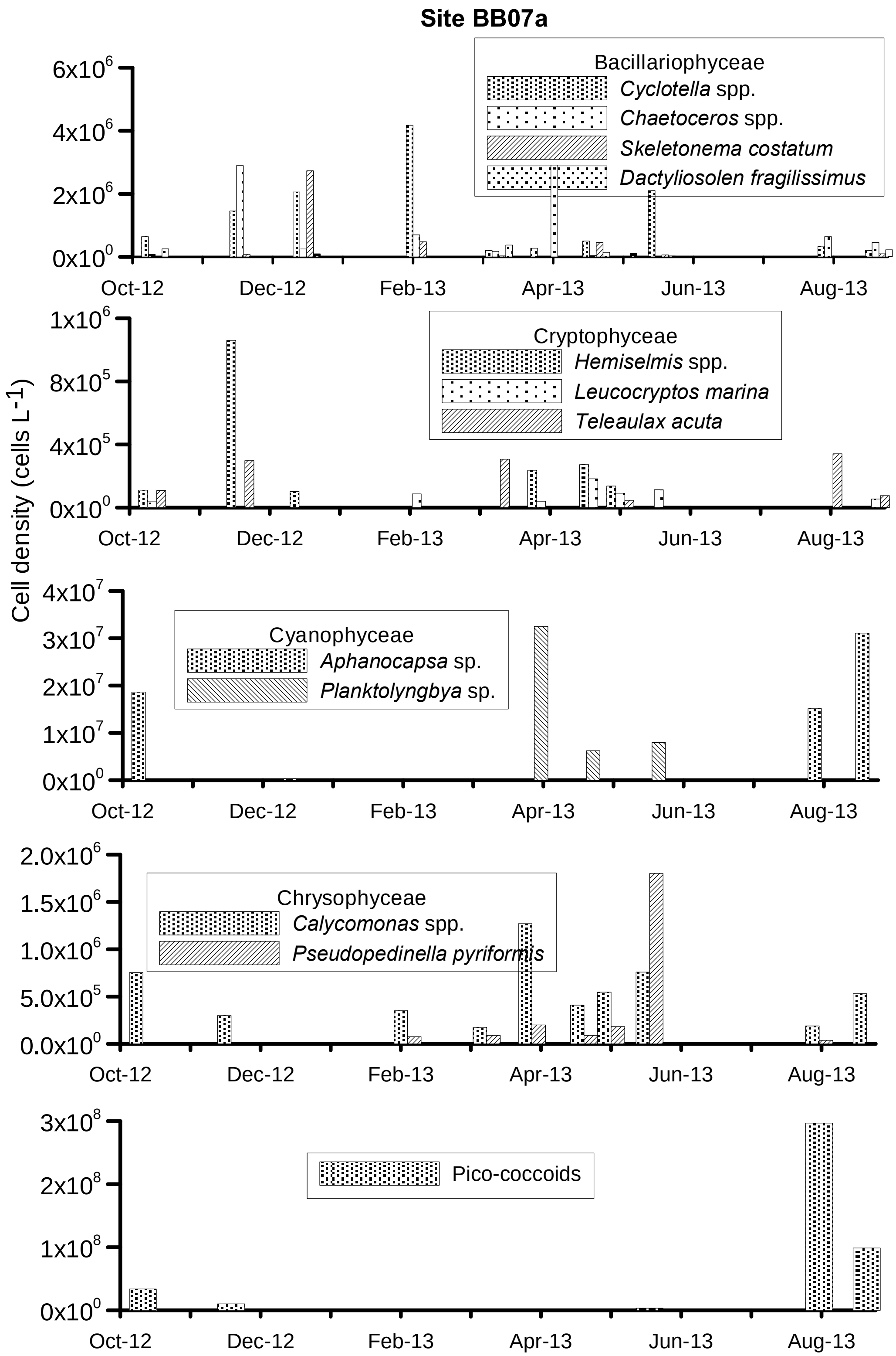


Fig. 6. Abundance and seasonal changes of some dominant species at site BB07a from October 2012 to August 2013.

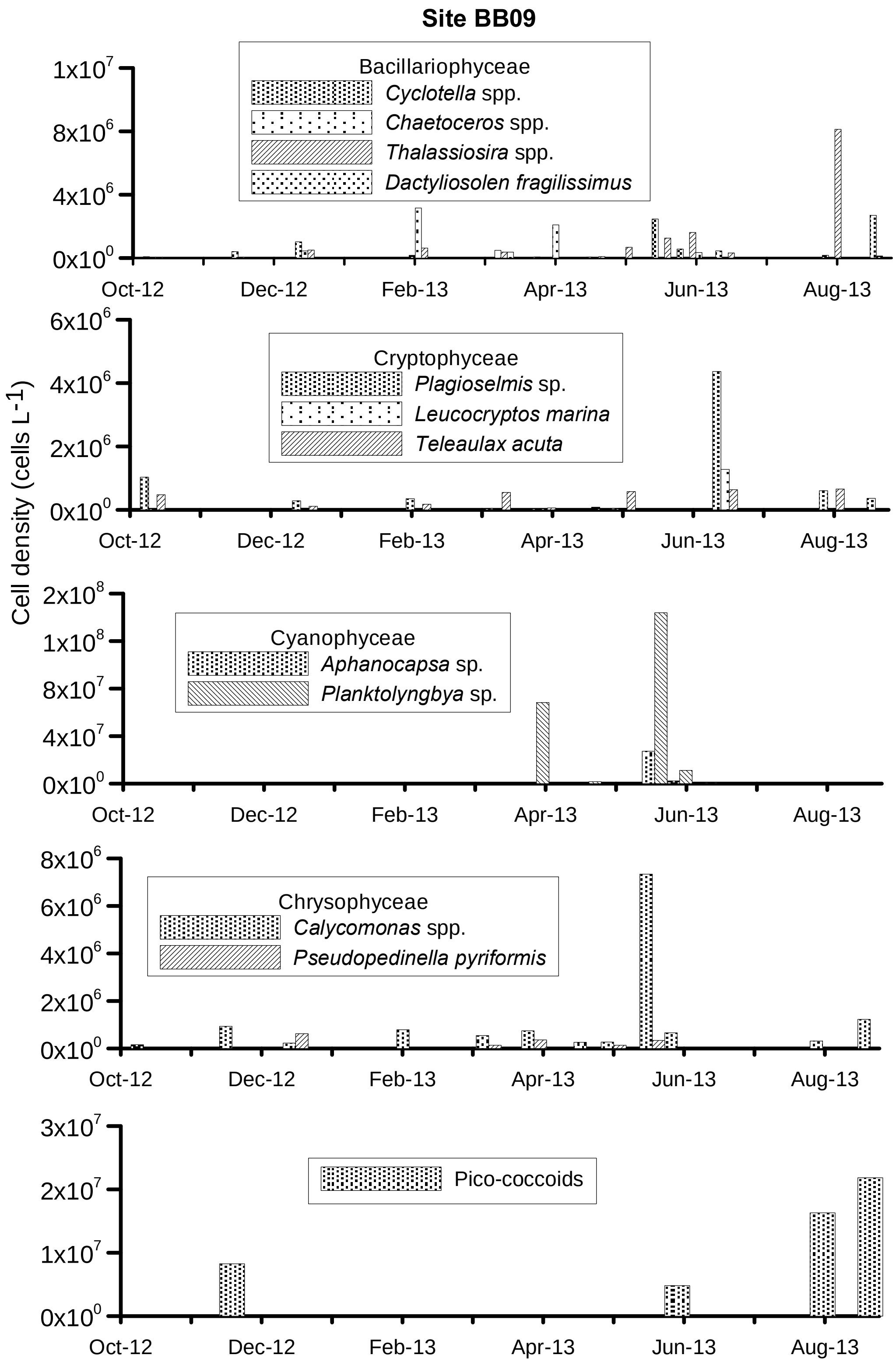


Fig. 7. Abundance and seasonal changes of some dominant species at site BB09 from October 2012 to August 2013.

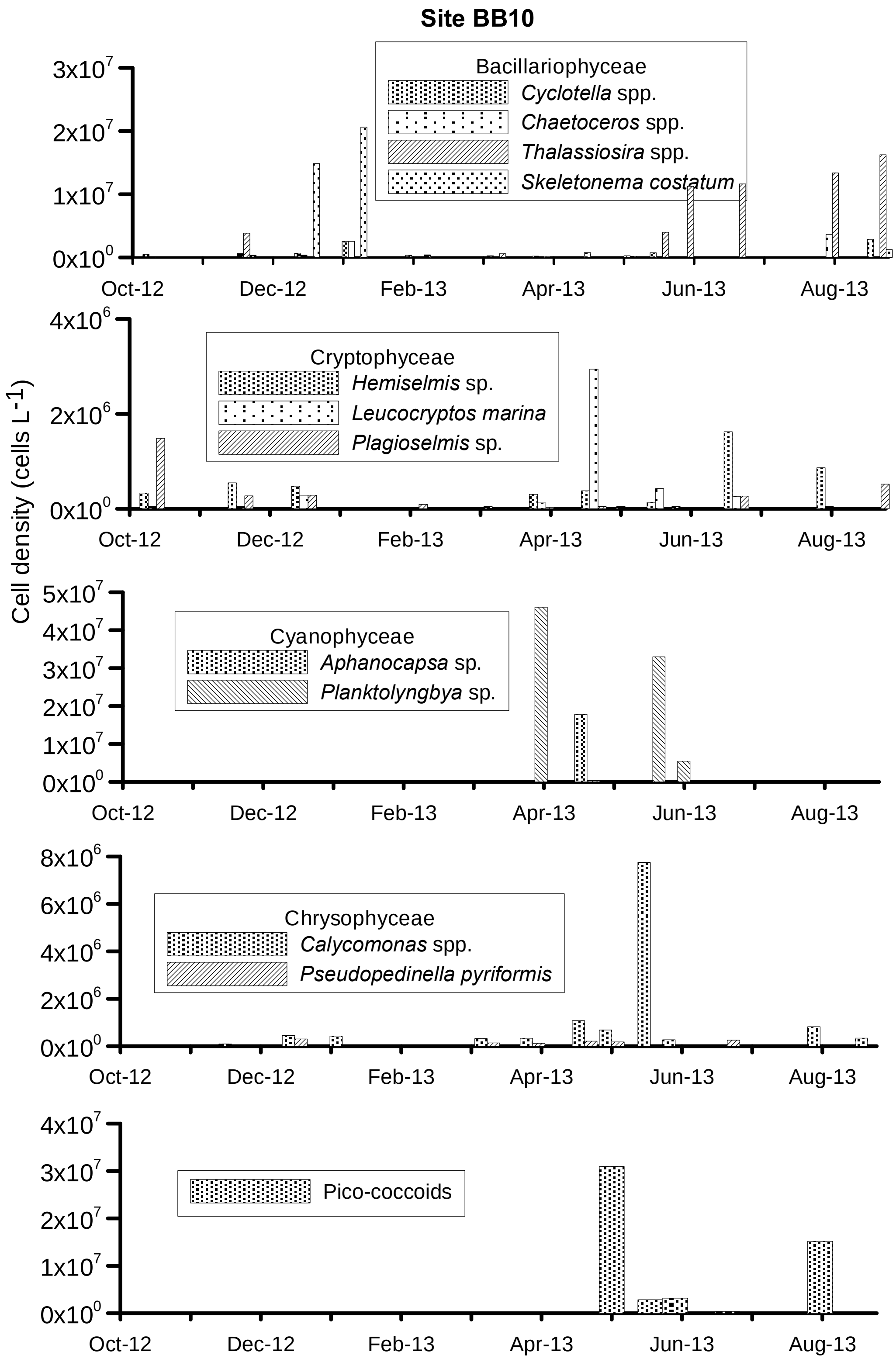


Fig. 8. Abundance and seasonal changes of some dominant species at site BB10 from October 2012 to August 2013.

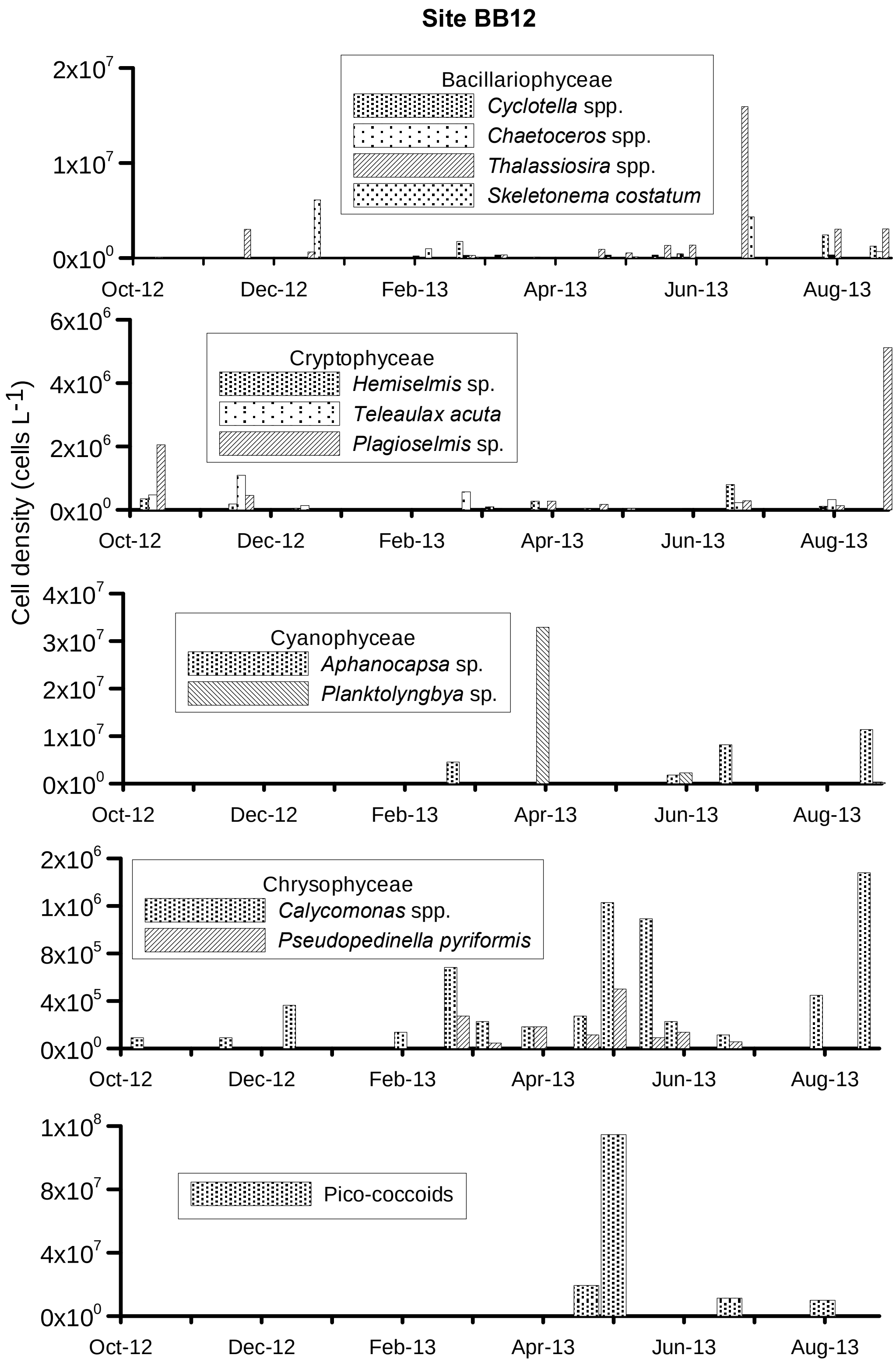


Fig. 9. Abundance and seasonal changes of some dominant species at site BB12 from October 2012 to August 2013.

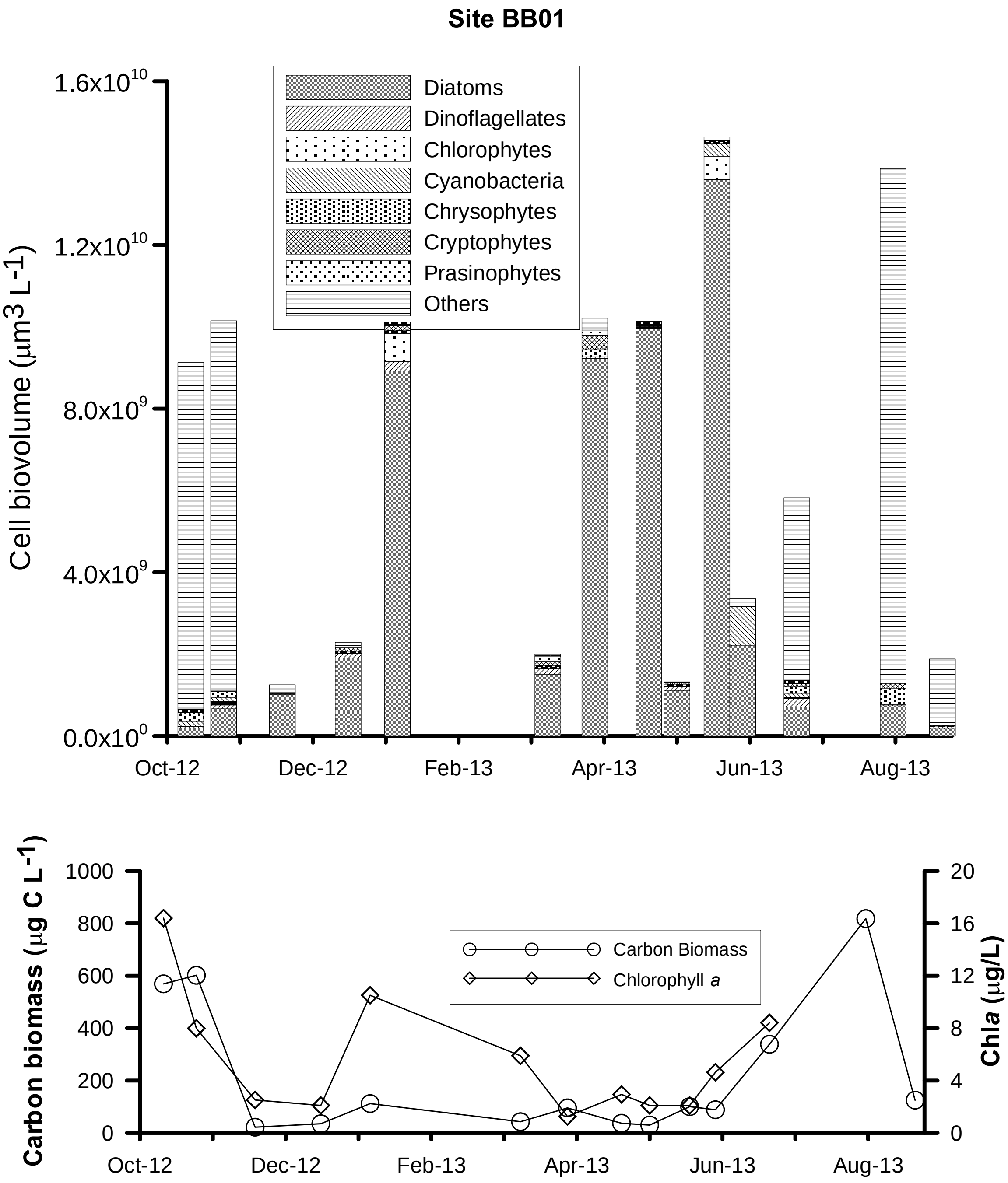


Fig. 10. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB01 from October 2012 to August 2013.

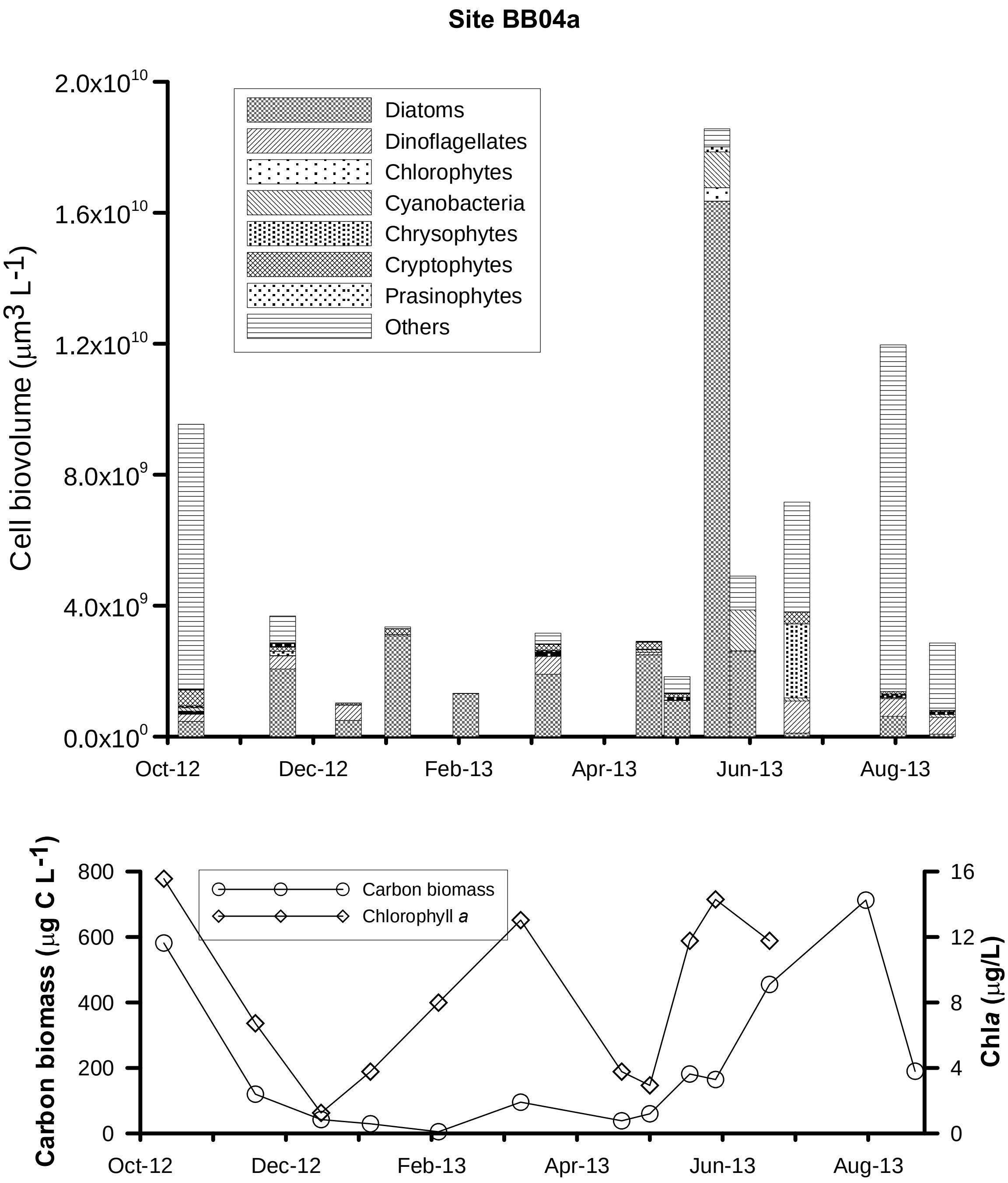
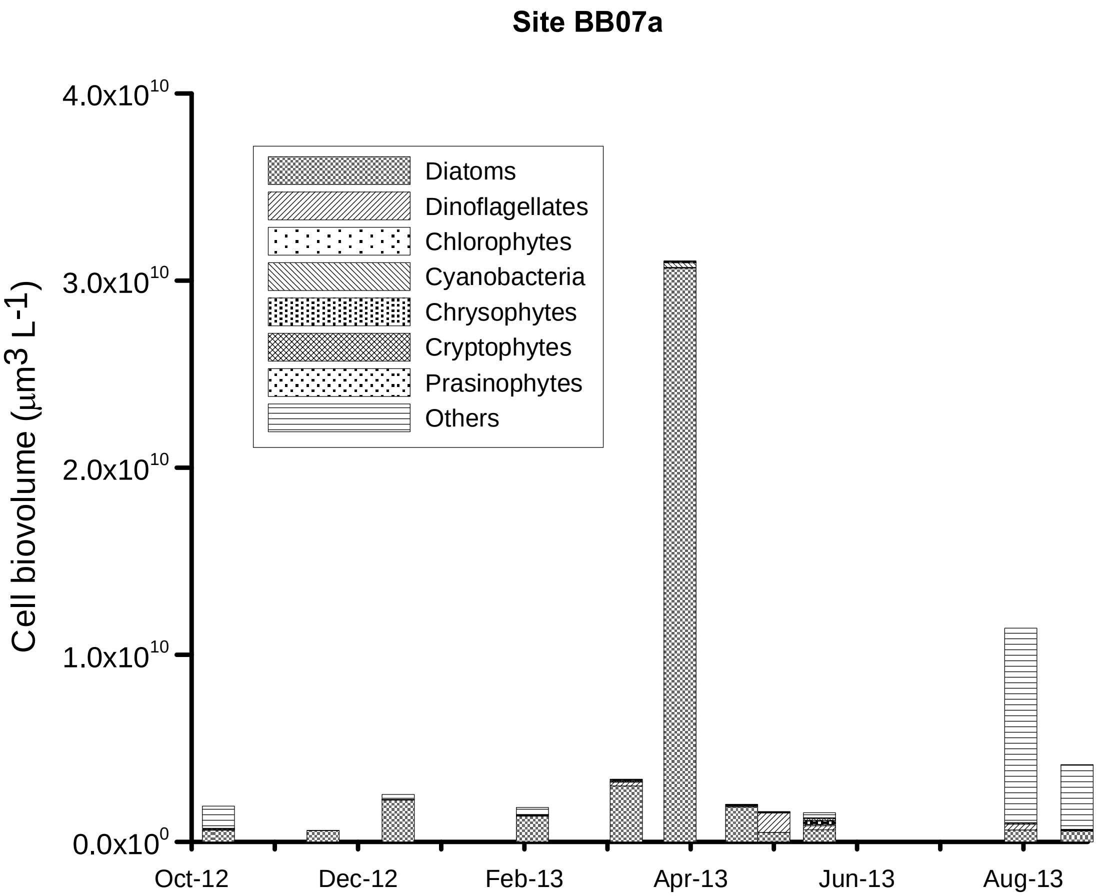


Fig. 11. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB04a from October 2012 to August 2013.



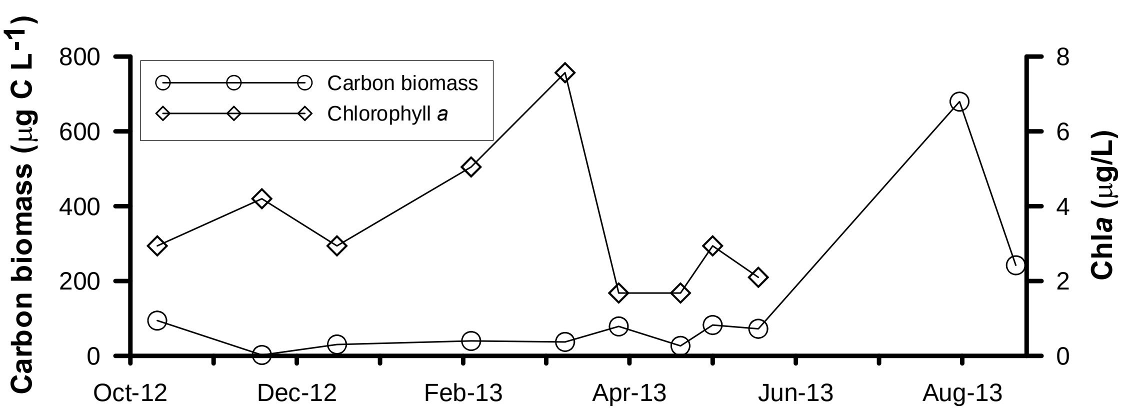


Fig. 12. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB07a from October 2012 to August 2013.

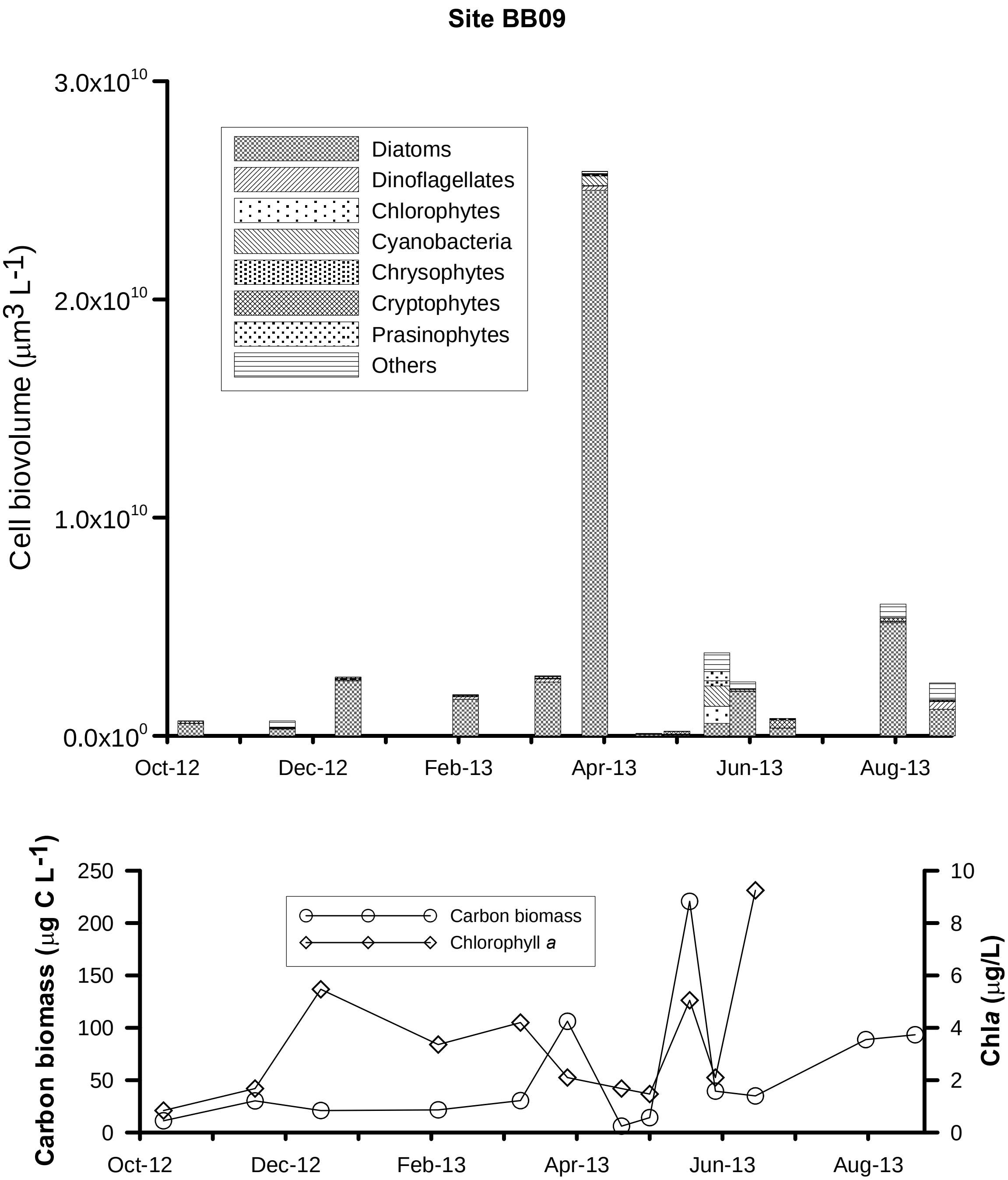


Fig. 13. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB01 from October 2012 to August 2013.

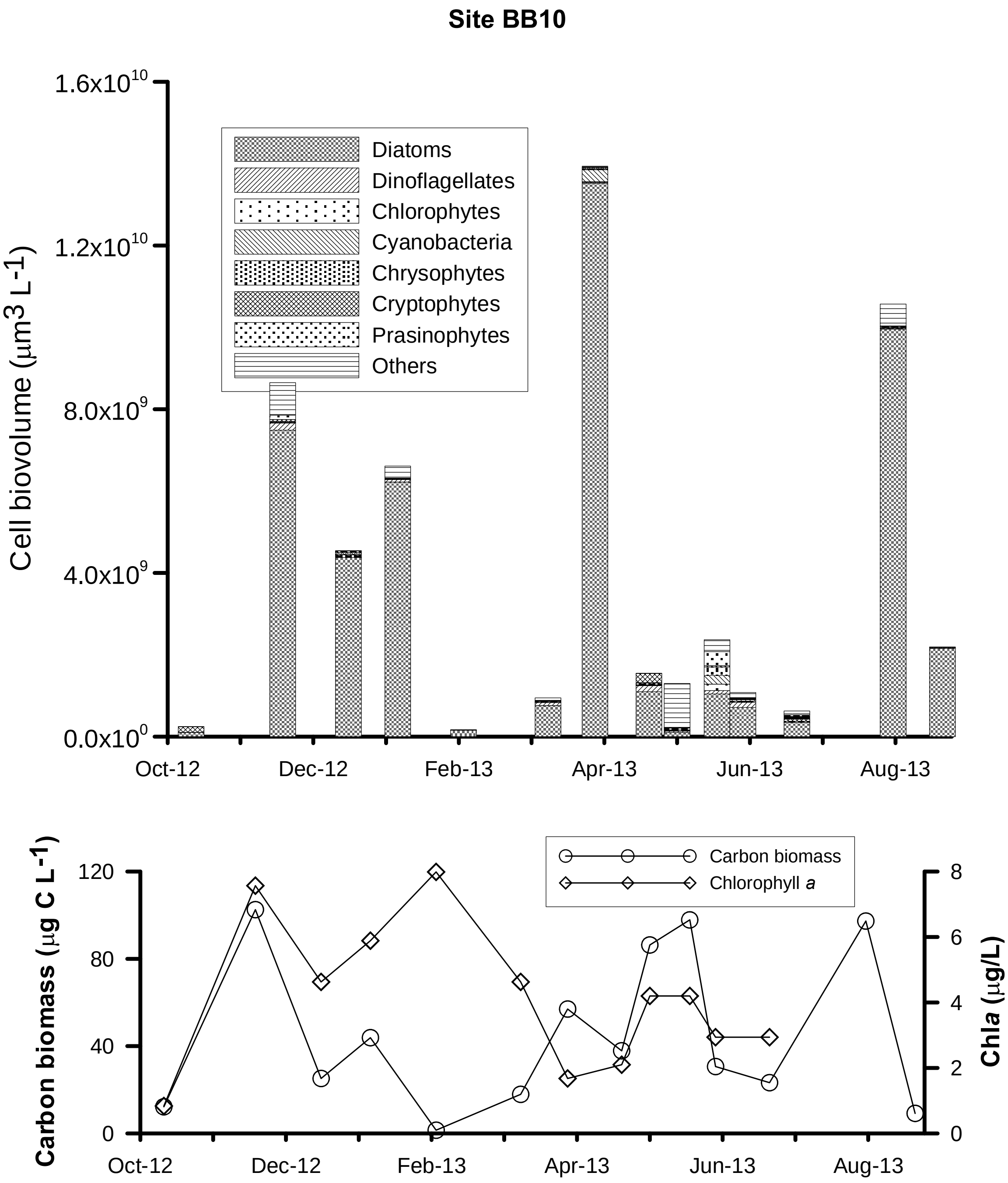


Fig. 14. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB10 from October 2012 to August 2013.

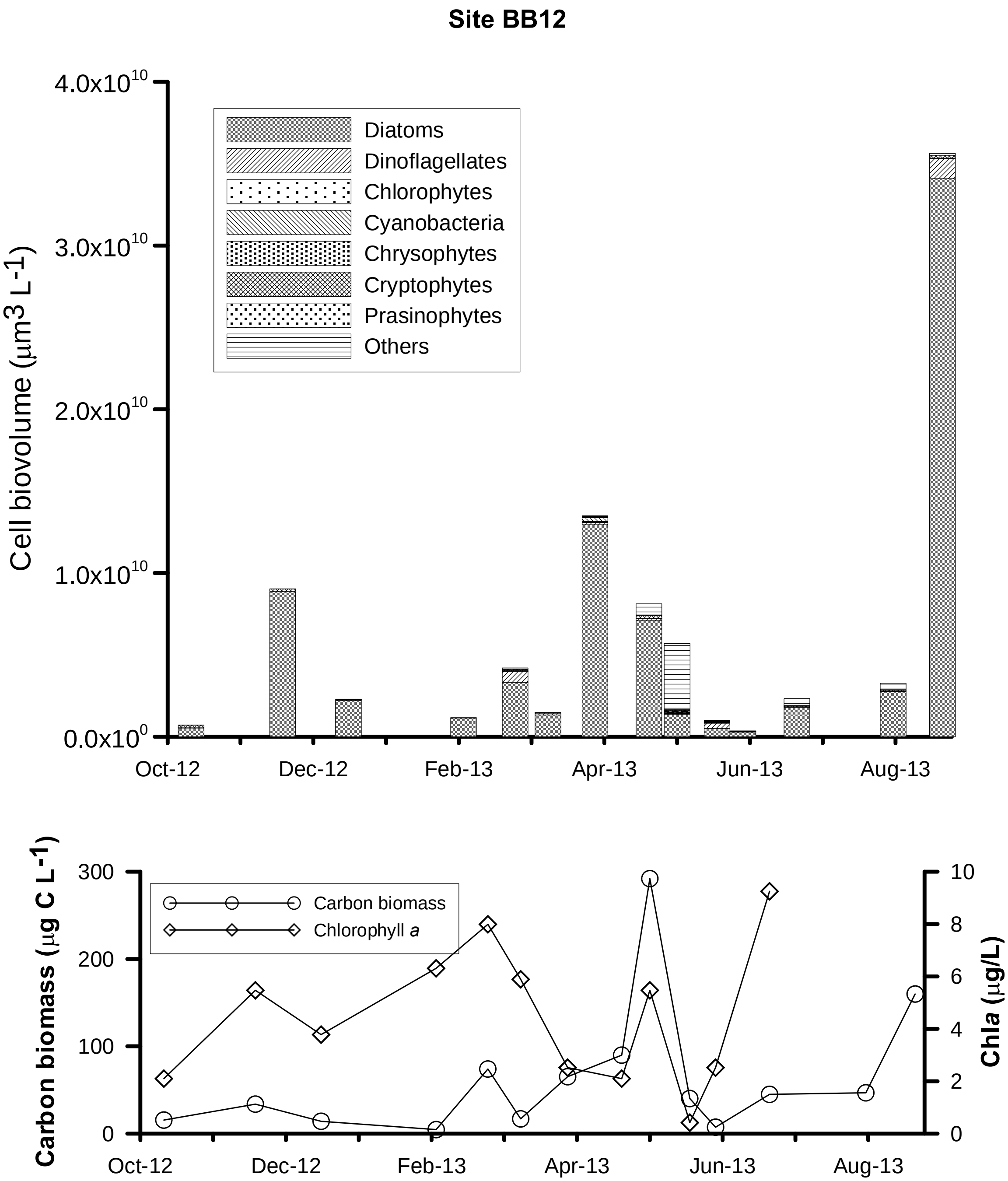


Fig. 15. Biovolume calculation and carbon biomass estimation of phytoplankton at site BB12 from October 2012 to August 2013.

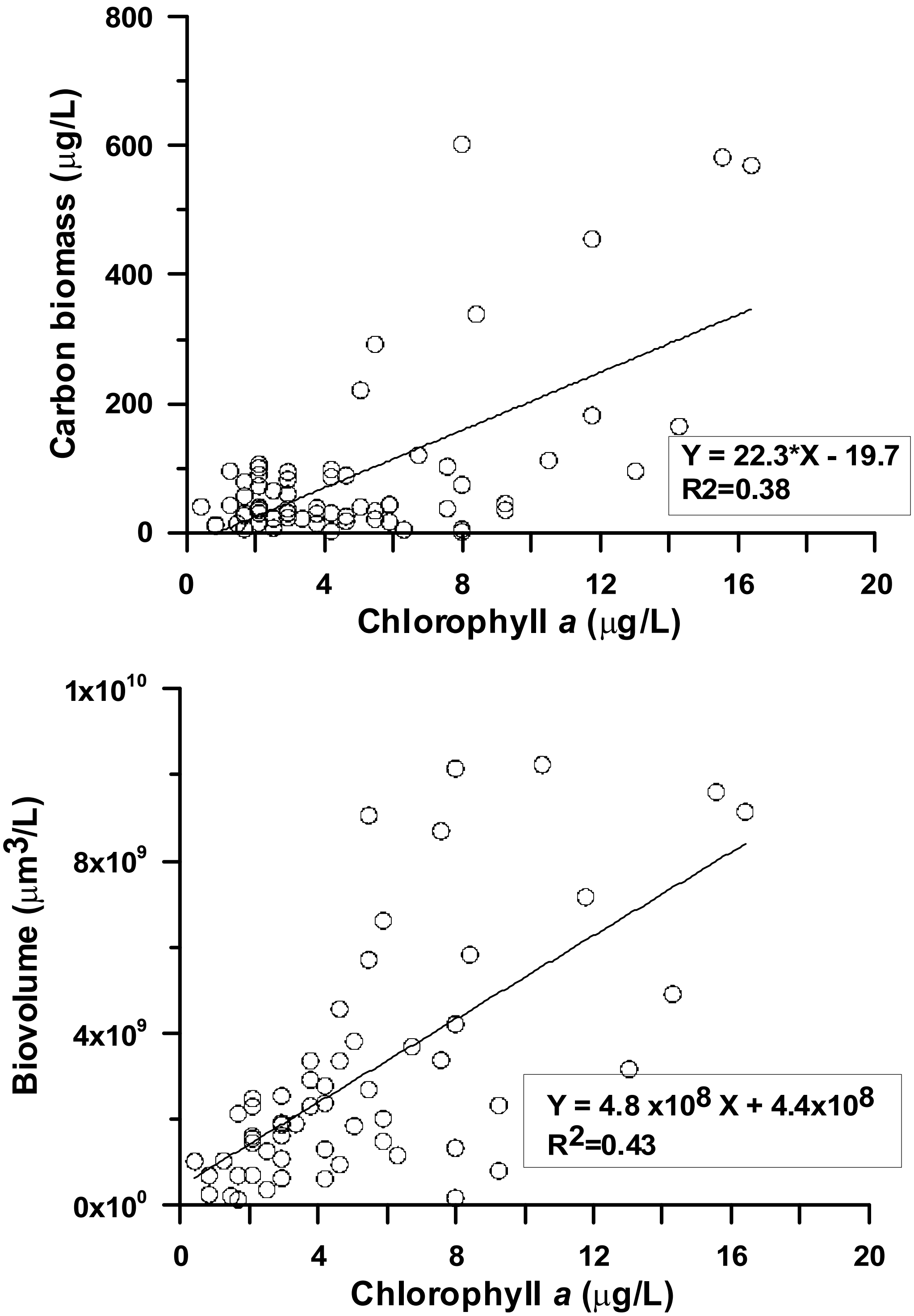


Fig. 16. Correlation of chlorophyll *a* and biovolume, and estimated carbon biomass based on phytoplankton community data from October 2012 to August 2013.

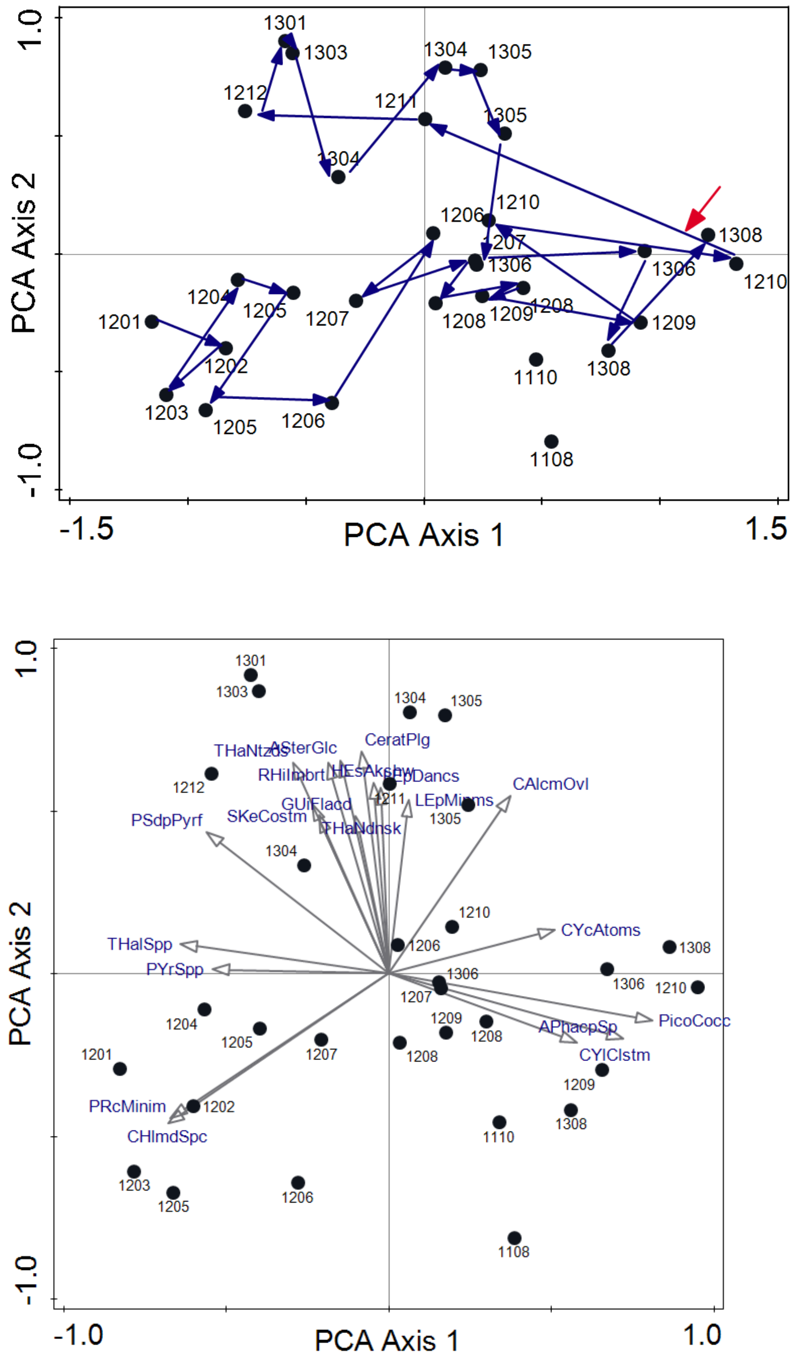


Fig. 17. Year-to-year changes of phytoplankton community at BB01 from August 2011 to August 2013. Each dot in the diagrams represents phytoplankton community in one sample. Samples labelled as collection year and month (YYMM). **Upper panel**: sample scatter diagram: the relative distance between samples reflects relative similarity in species composition; **Lower panel**: sample-species biplot, species arrows point to the direction of steepest increase of species values (see more detailed in Methods).

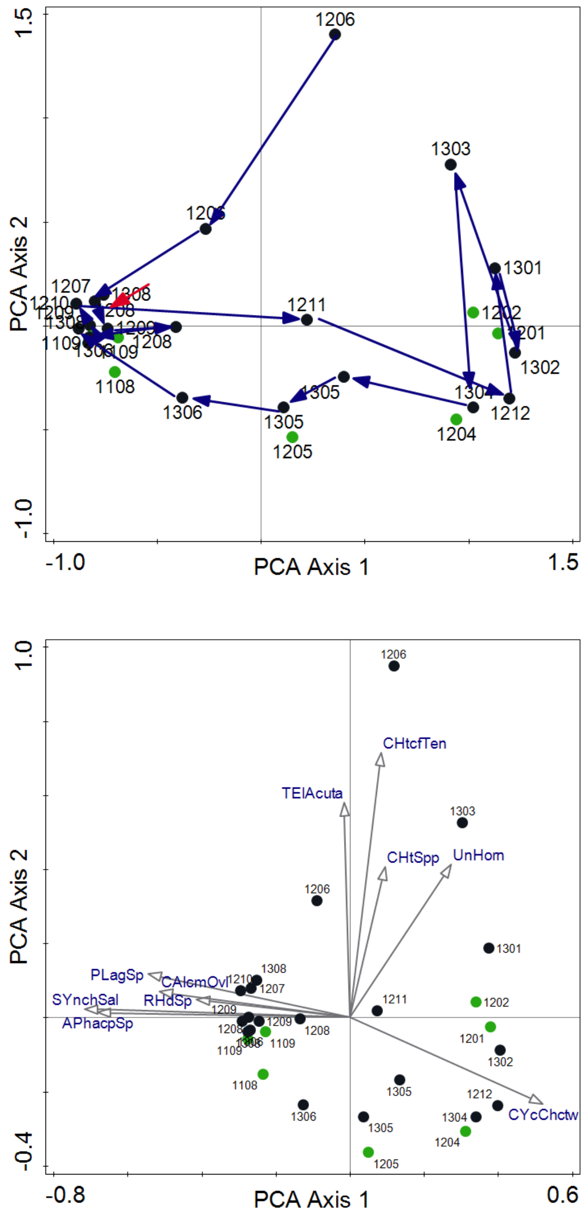


Fig. 18. Year-to-year changes of phytoplankton community at BB04a from August 2011 to August 2013. Each dot in the diagrams represents phytoplankton community in one sample. Samples labelled as collection year and month (YYMM). Upper panel: sample scatter diagram, the relative distance between samples reflects relative similarity in species composition; Lower panel: sample-species biplot, species arrows point to the direction of steepest increase of species values (see more detailed in Methods).

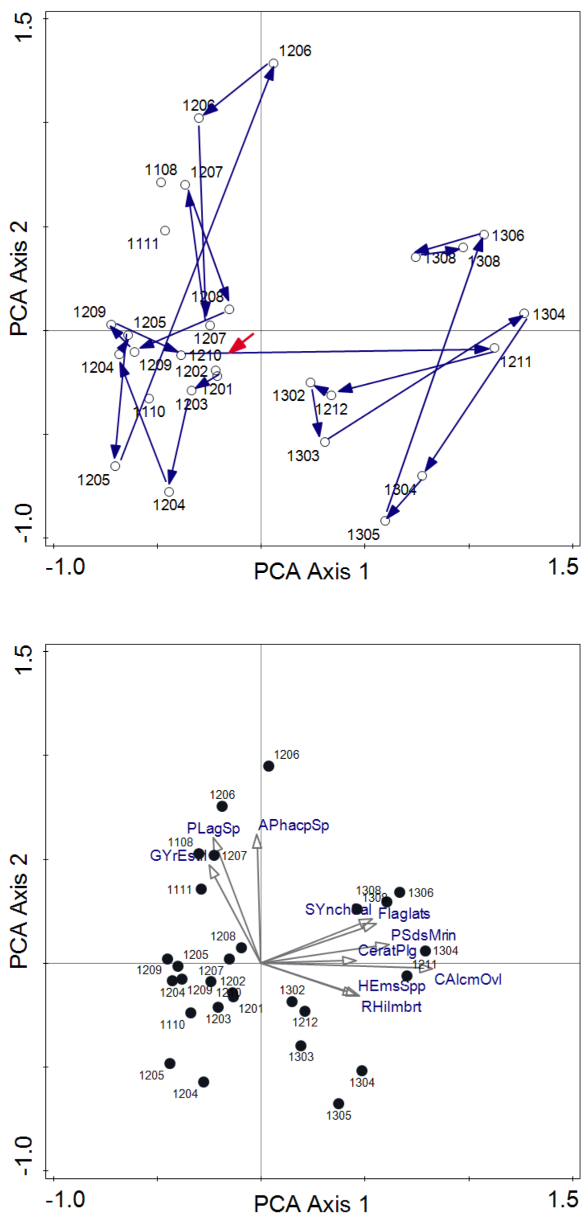


Fig. 19. Year-to-year changes of phytoplankton community at BB09 from August 2011 to August 2013. Each dot in the diagrams represents phytoplankton community in one sample. Samples labelled as collection year and month (YYMM). **Upper panel**: sample scatter diagram: the relative distance between samples reflects relative similarity in species composition; **Lower panel**: sample-species biplot, species arrows point to the direction of steepest increase of species values (see more detailed in Methods).

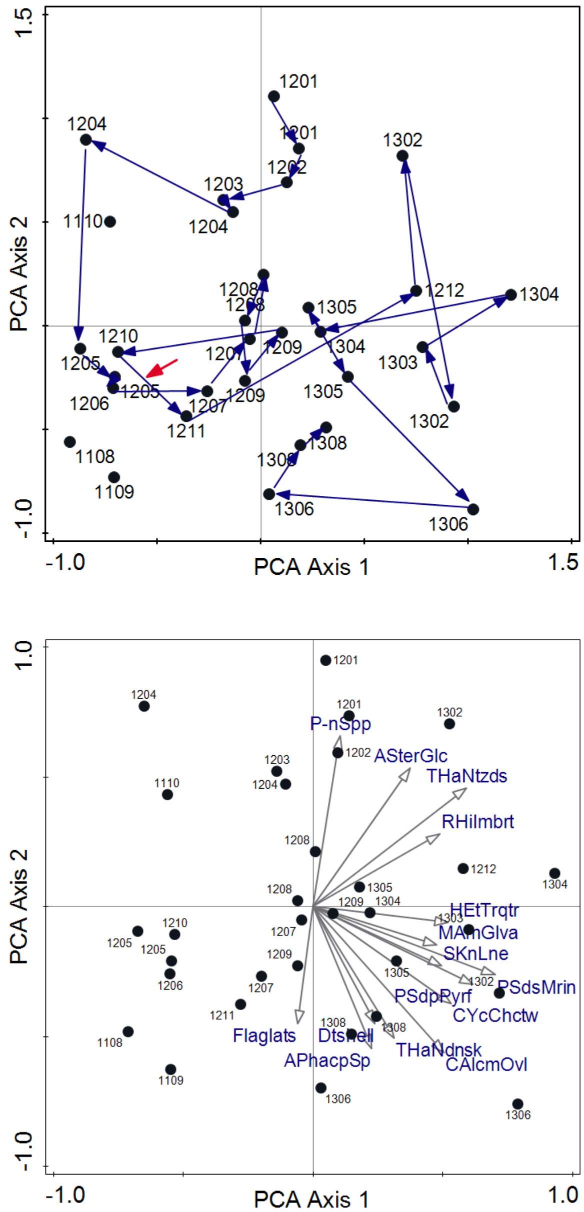


Fig. 20. Year-to-year changes of phytoplankton community at BB12 from August 2011 to August 2013. Each dot in the diagrams represents phytoplankton community in one sample. Samples labelled as collection year and month (YYMM). **Upper panel**: sample scatter diagram: the relative distance between samples reflects relative similarity in species composition; **Lower panel**: sample-species biplot, species arrows point to the direction of steepest increase of species values (see more detailed in Methods).

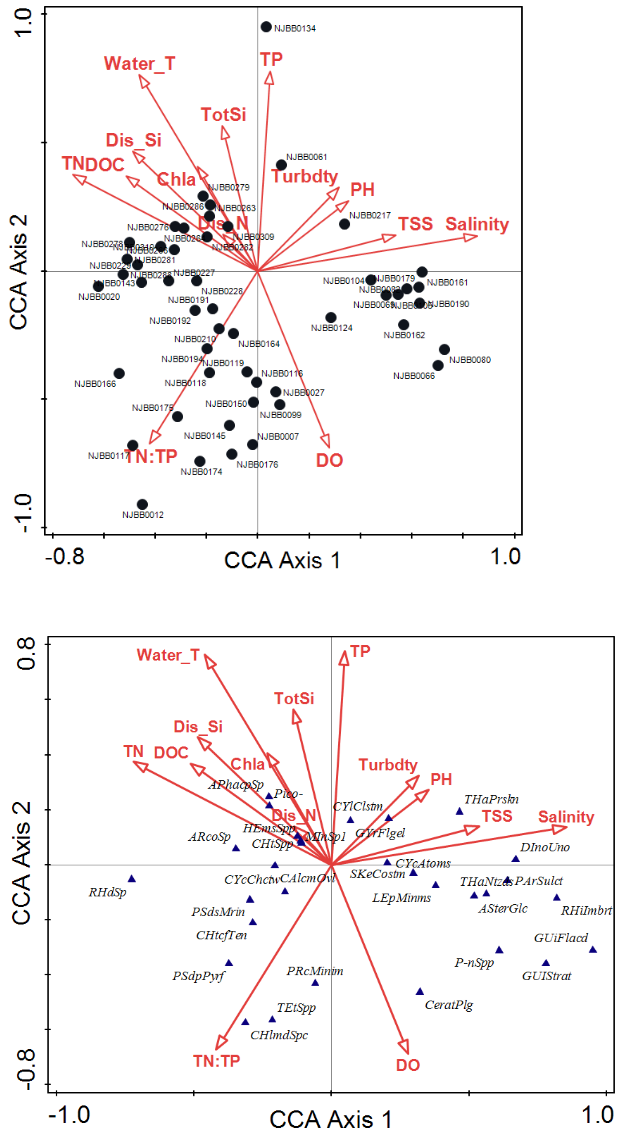


Fig. 21. Results of canonical correspondence analysis (CCA) based on year-one phytoplankton data collected from August 2011 to September 2012. **Upper panel**: samples-environmental variables biplot showing the changes of the phytoplankton community of 134 samples in environmental conditions; **Lower panel**: species-environmental variables biplot showing changes in species composition explained by environmental conditions. The first 30 species with highest weight are shown.

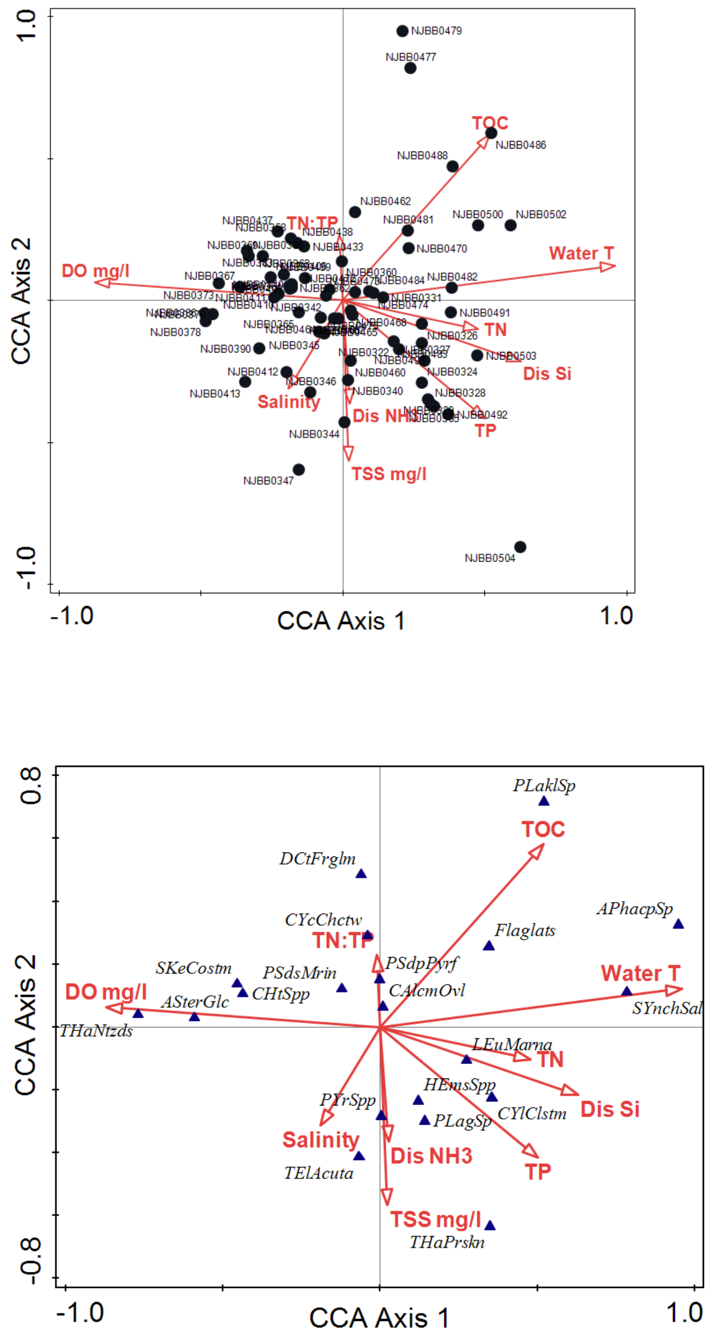


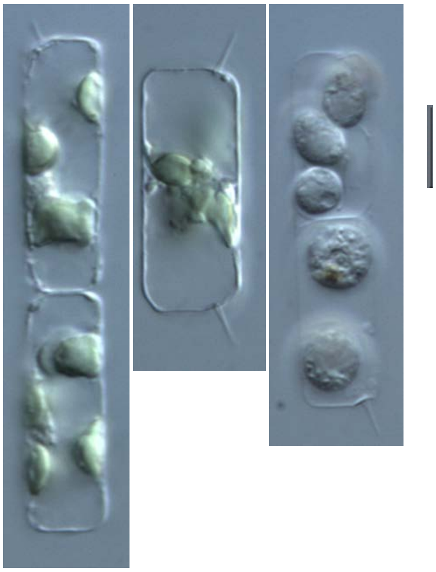
Fig. 22. Results of canonical correspondence analysis (CCA) based on year-two phytoplankton data collected from October 2012 to August 2013 at six sites. **Upper panel**: samples-environmental variables biplot showing the changes of the phytoplankton community of 67 samples in environmental conditions; **Lower panel**: species-environmental variables biplot showing changes in species composition explained by environmental conditions. The first 20 species with highest weight are shown.

APPENDICES

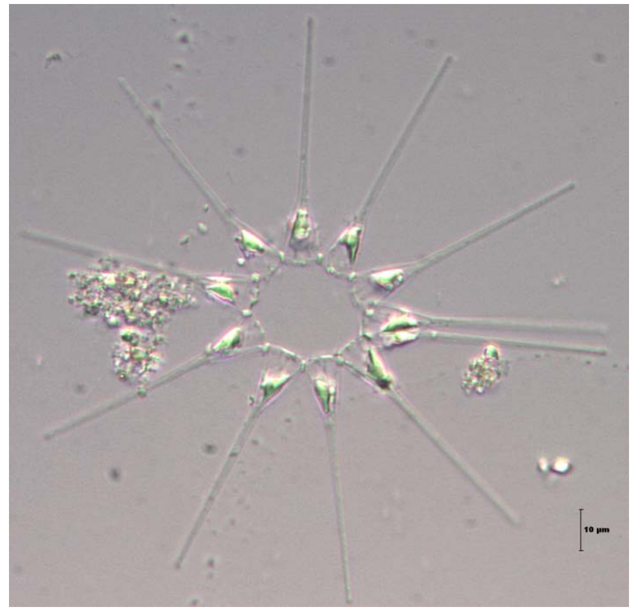
Appendix 1(CD): Plates: Image documentation on some abundant phytoplankton species.

Appendix 2 (CD): Excel files with data on phytoplankton species cell density, biovolume calculation and carbon estimation for sites BB01, BB04a, BB07a, BB09, BB10, and BB12 from October 2012 to August 2013.

Plate 1 Diatoms



Dactyliosolen fragilissimus



Asterionellopsis glacialis

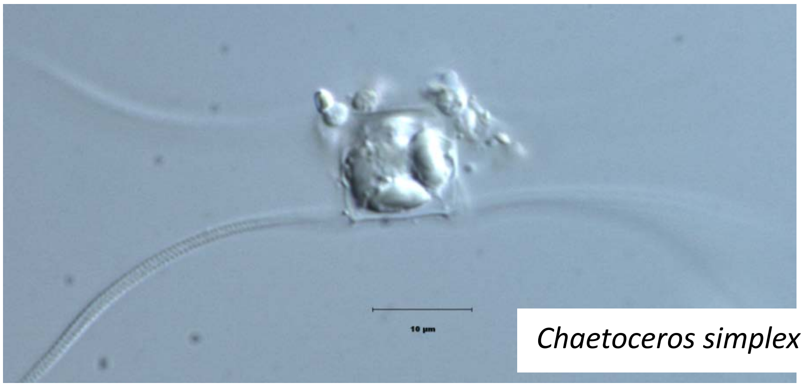
Cyclotella choctawatcheena

Cyclotella atomus

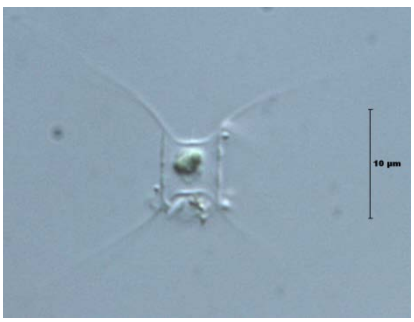
Cylindrotheca closterium

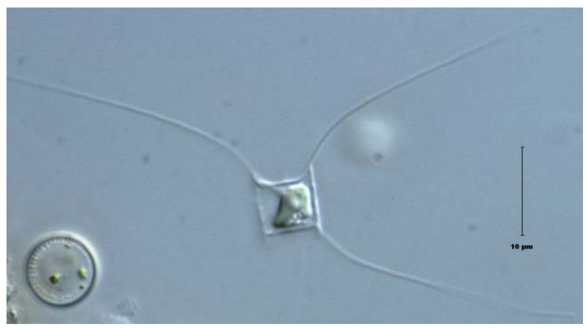
Plate 2 Diatoms

Chaetoceros spp.



Chaetoceros simplex

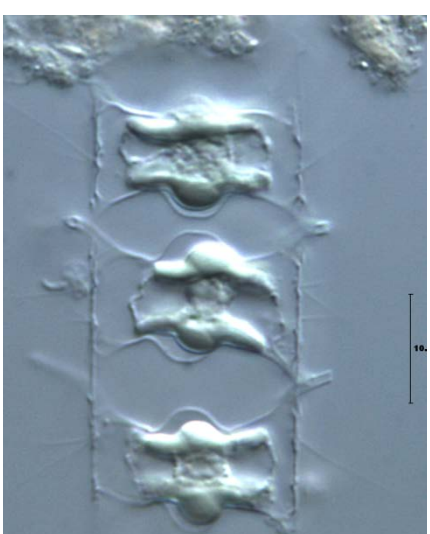




Chaetoceros tenuissimus

Ch. Subtilis var. abnormis fo. simplex

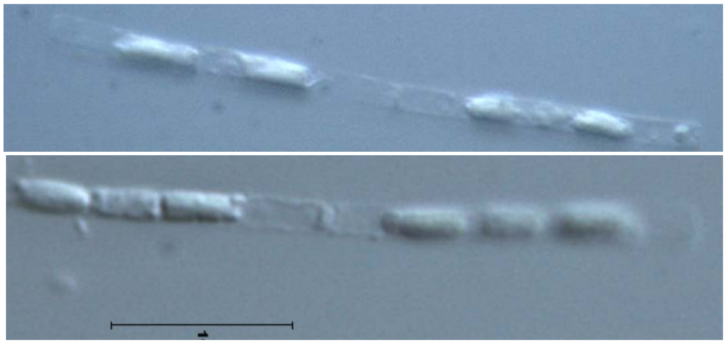
Ch. danics



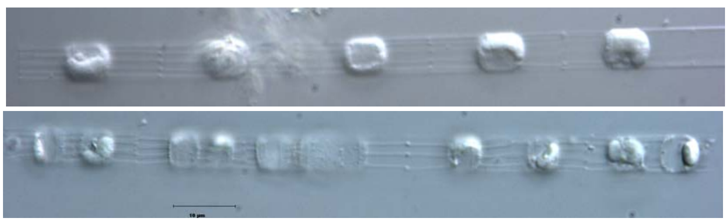
Attheya decora

Ch. didymus

Plate 3   Diatoms

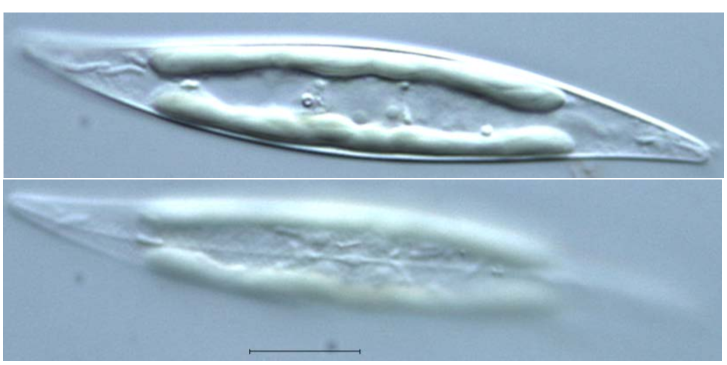


Leptocylindrus minimus



Skeletonema “costatum’

Pseudo-nitzschia pungens

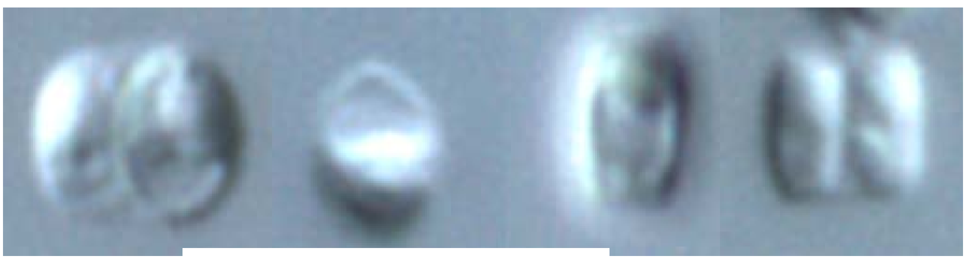


Pleurosigma salinarum

Rhizosolenia sp.

Plate 4 Diatoms

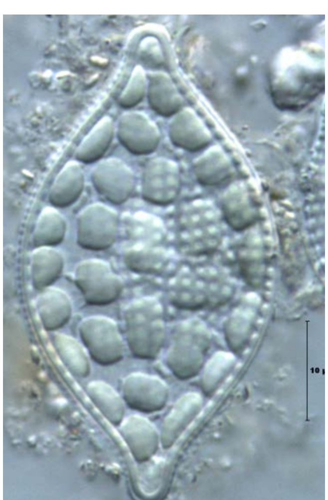
Minidiscus spp.



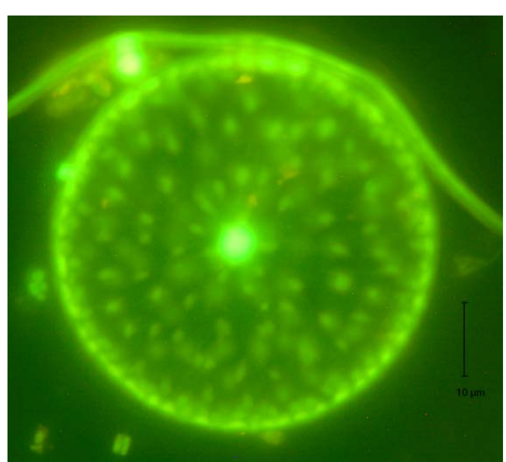
PhaeodactylumQ tricornutum

Minutocellus scriptus

Thalassionema nitzschioides



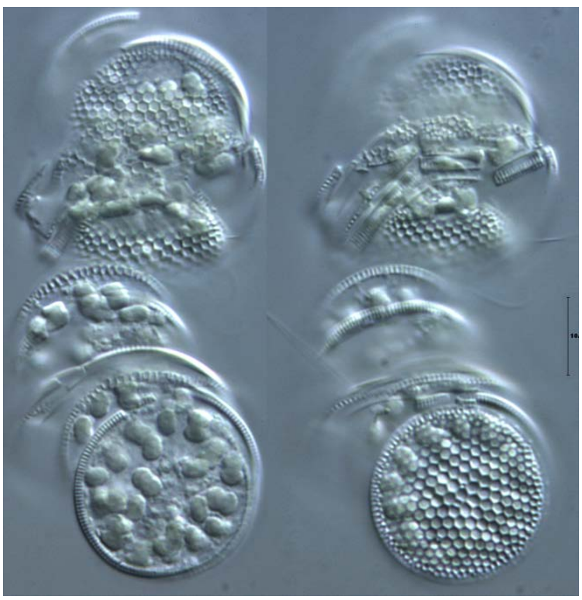
Rhaphoneis amphiceros



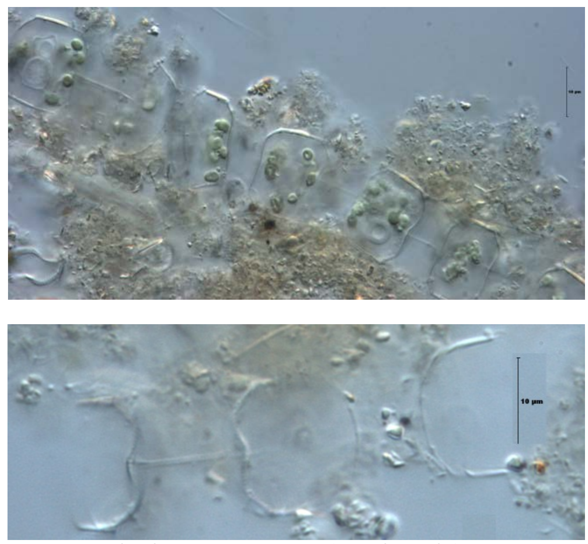
Coscinodiscus concinnus

Plate 5 Diatoms

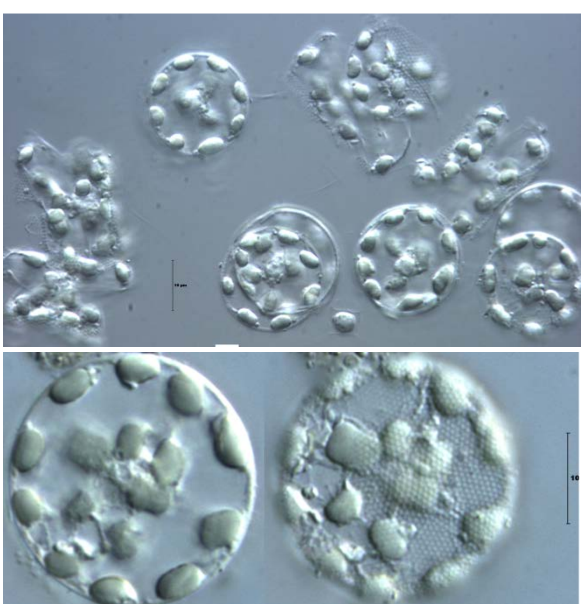
Thalassiosira proschkinae



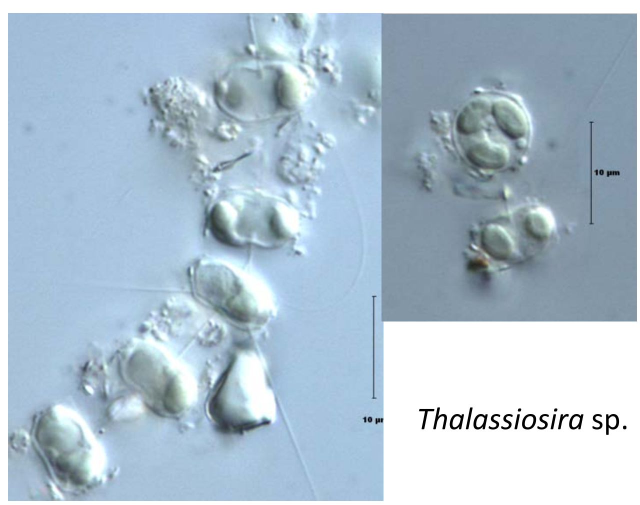
Thalassiosira oestrupii



Thalassiosira nordenskioeldii

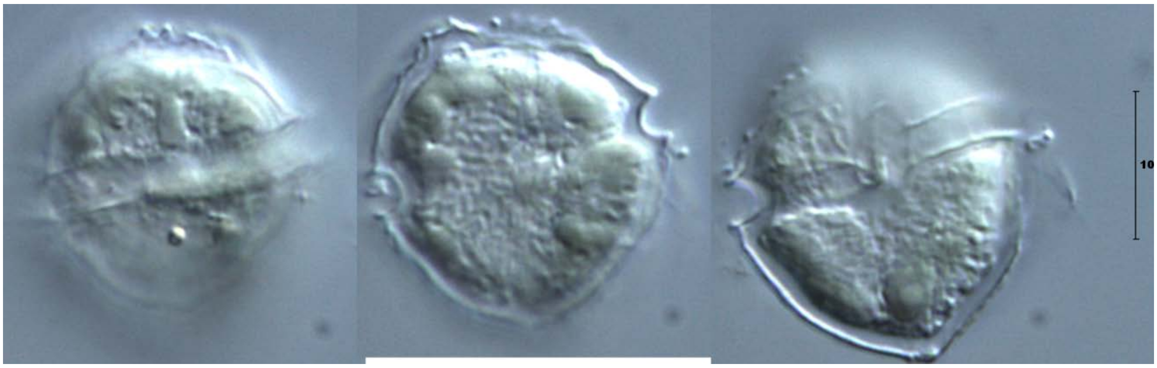


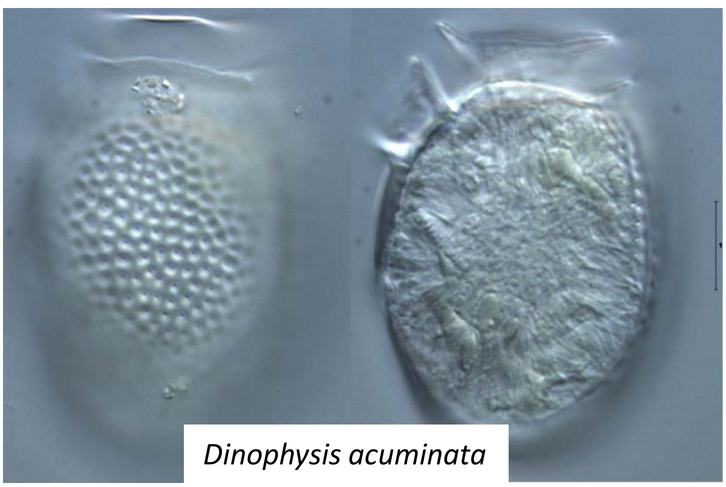
Thalassiosira tenera



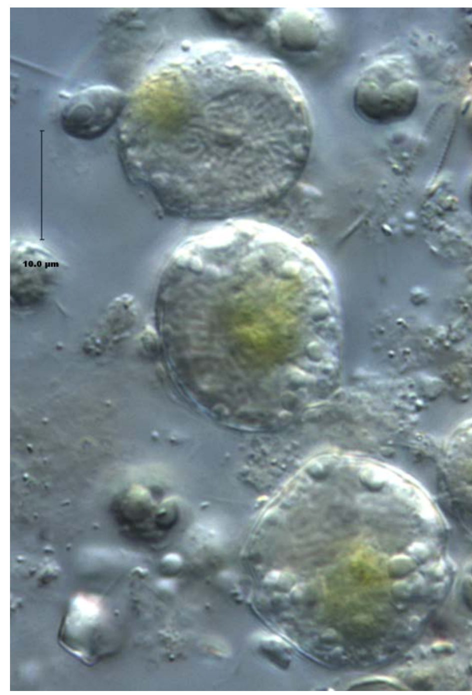
Thalassiosira sp.

Plate 6   Dinoflagellates





Dinophysis acuminata



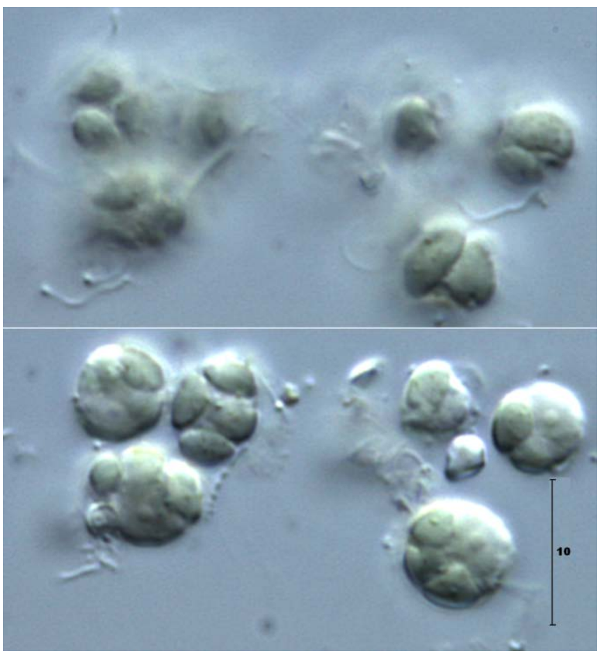
Gyrodinium flagellare

Alexandrium tamarense

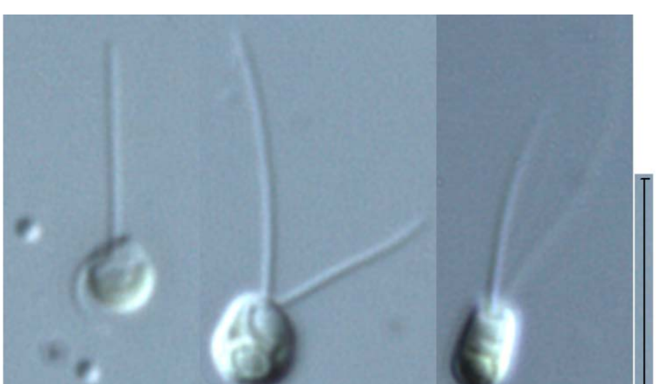
Heterocapsa rotundata

Plate 7 Phytoflagellates

Heterosigma akashiwo

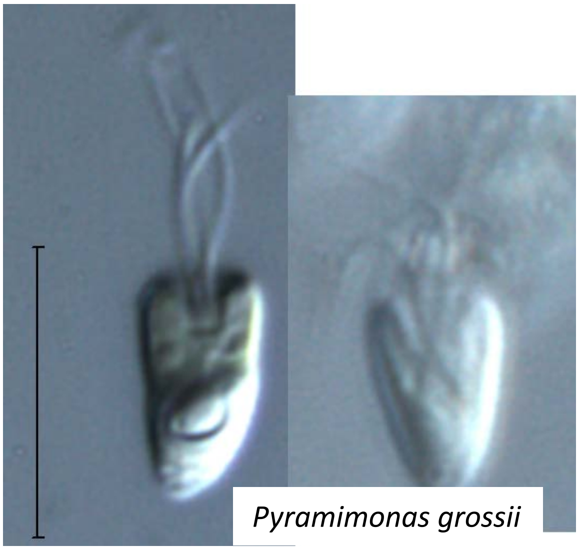


Pseudopedinella pyriformis

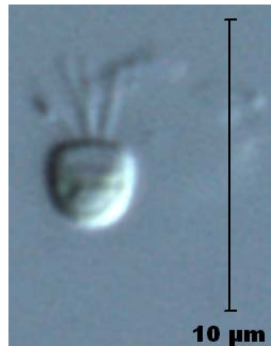


Pseudoscourfieldia marina

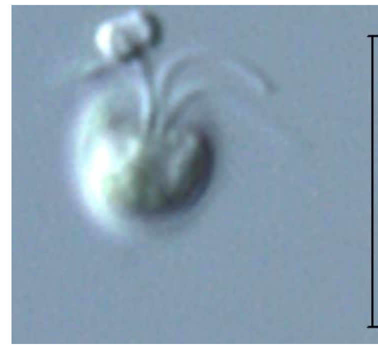
Plate 8 Phytoflagellates



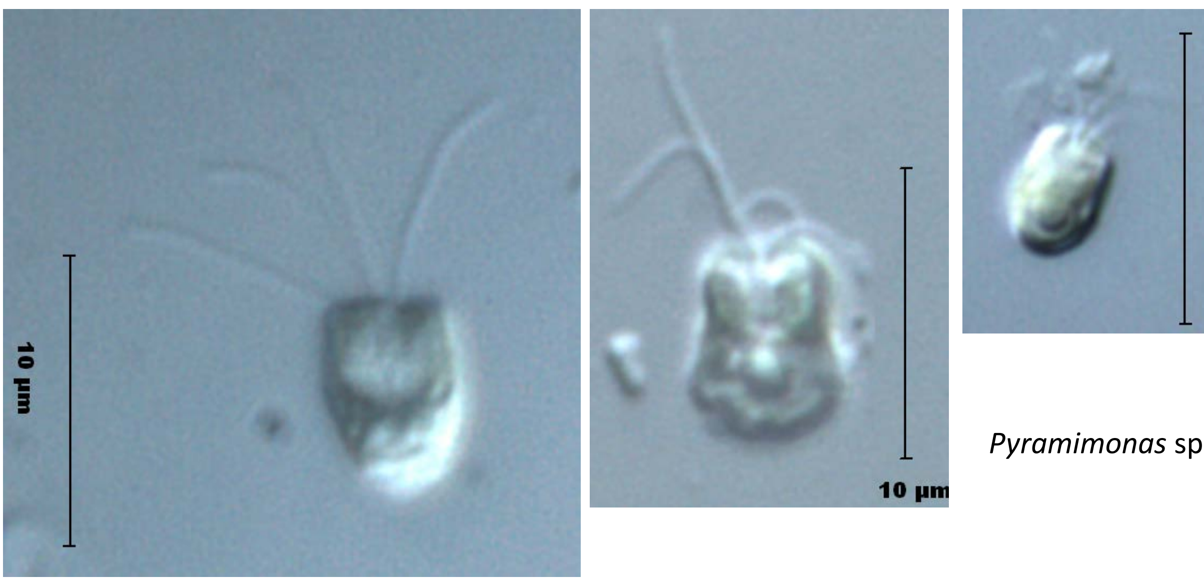
Pyramimonas grossii



Pyramimonas virginica

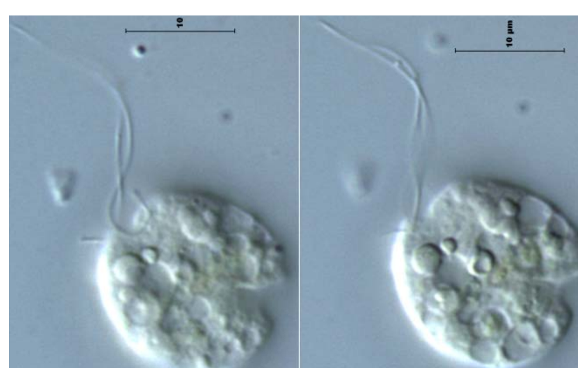


Pyramimonas orientalis



Pyramimonas spp.

Mamiella gilva



Pachysphaera pelagica